Loss of endothelium-dependent vasodilatation and nitric oxide release after myocardial protection with University of Wisconsin solution

University of Wisconsin solution has proved to be a superior form of cardioplegia for cardiac transplantation, demonstrating better functional recovery than that provided by extracellular crystalloid solutions. Furthermore, experimental data have suggested a role for University of Wisconsin solution in protection of the neonatal heart during operations for congenital heart defects. However, significant concerns have been raised regarding potential endothelial injury from the high potassium concentration contained in University of Wisconsin solution that could affect its safety and thus its clinical application. Fourteen neonatal (aged 1 to 3 days) piglet hearts were harvested and supported on an isolated, blood-perfused circuit. Endothelium-dependent vasodilatation was measured by bradykinin (10^{-6} mol/L) infusion and nitric oxide release was determined. Endothelium-independent vasodilatation was then induced by sodium nitroprusside (10^{-6} mol/L) infusion. A 2-hour period of cold cardioplegic arrest was instituted with multidose University of Wisconsin solution (group 1, $n = 7$) or blood cardioplegia (group 2, $n = 7$). After reperfusion and stabilization, another stimulation with bradykinin and nitroprusside was carried out and nitric oxide was again measured. After 2 hours of arrest with University of Wisconsin solution, there was a near-complete loss of vasodilatation in response to bradykinin infusion; coronary blood flow reached 245 % of baseline before arrest versus only 117% of baseline after arrest ($p = 0.0011$). This correlated with an inability of the endothelium to release nitric oxide (96 ± 30 nmol/min before arrest versus -32 ± 9 nmol/min after arrest, $p = 0.0039$). In group 2, the vasodilatory response to bradykinin was preserved after arrest and reperfusion; 265 % of baseline before arrest versus 222 % of baseline after arrest. These results demonstrate a loss of endothelium-dependent vasodilatation after multidose University of Wisconsin cardioplegia caused by the inability of the endothelium to release nitric oxide. In contrast, blood cardioplegia does not result in impaired endothelial function. (J THORAC CARDIOVASC SURG 1994;107:257-64)

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University of Wisconsin (UW) solution has proved to be a superior agent in organ preservation.¹⁻³ Studies in cardiac transplantation have demonstrated superior functional recovery with UW solution compared with extracellular crystalloid solutions.⁴⁻⁶ In view of these reports, many centers (including our own) have adopted UW solution as the cardioplegic solution of choice for cardiac preservation. Furthermore, because of UW solution's clear superiority in myocardial protection for transplantation, it has been suggested that UW solution may have a role in myocardial protection for purposes other than transplantation. In particular, UW solution could be

Table I. Composition of cardioplegic solutions

Cardioplegia	Group 1 (UW solution)	Group 2 (blood cardioplegia)
Sodium (mEq/L)	132	133
Potassium (mEq/L)	22/10	140
Chloride (mEq/L)	93	120
Calcium (mmol/L)	0.6	Trace
Osmolarity (mOsm)	333	320
pH	7.6	7.4
Pentafracton (gm/L)	50	
Lactobionic acid (gm/L)	35.8	
Raffinose pentahydrate (gm/L)	17.8	
Adenosine (gm/L)	1.34	
Allopurinol (gm/L)	0.136	
Glutathione (gm/L)	0.922	

applied to the protection of the immature myocardium, which remains suboptimal with current techniques.⁷⁻¹⁰

However, increased recognition of the importance of endothelial physiology in the regulation of coronary blood flow,¹¹⁻¹⁵ platelet aggregation,¹⁶⁻¹⁸ and prevention of atherosclerosis¹⁸ has raised concern regarding potential endothelial injury from the high potassium concentration in UW solution. Loss of vasodilatory reserve has been demonstrated after arrest and storage with high-potassium cardioplegia solutions,^{17,19-23} including UW solution.²⁴ Although functional endothelial injury has been implicated as the cause of the impaired vasodilatory response,¹⁸ the specific pathways involved, including the role of nitric oxide, have been poorly defined.

Materials and methods

Hearts were removed from 14 neonatal (1 to 3 days old) piglets (Duroc) without intervening ischemia and placed on an isolated, blood-perfused circuit. Hearts were perfused with oxygenated arterial blood from a support animal warmed to 38° C by a blood cardioplegia device (BCD; Shiley, Inc., Irvine, Calif.). The blood was delivered retrogradely through the innominate artery to the aortic root at a constant pressure of 80 mm Hg.¹⁰ Hearts were not placed in the working mode so that accurate coronary arterial and venous samples could be obtained.

After assessment of baseline coronary blood flow (collected from the pulmonary artery catheter), vasodilation was induced by instilling bradykinin mixed in blood to a final concentration of 10⁻⁶ mol/L. A 1-minute infusion was given and coronary blood flow per minute was measured for 3 minutes. The average coronary blood flow measured during this period was calculated and expressed both in milliliters per gram of heart tissue and as a percentage of baseline coronary flow. New baseline flows were obtained before each intervention. In addition, a 5 ml sample of arterial and coronary sinus blood was obtained for nitric oxide determination after the 1-minute infusion of bradykinin.

After return of coronary flow to a steady baseline, usually 30

minutes after the bradykinin infusion, blood containing sodium nitroprusside (10⁻⁶ mol/L) was infused for 1 minute and coronary blood flow was measured for 3 minutes. Once again, the average coronary blood flow per minute during this 3-minute period was used for comparison and results were expressed as percentage of baseline coronary flow. The perfusion pressure was kept constant at 80 mm Hg throughout both series of experiments.

Hearts were then arrested with either UW solution (group 1, *n* = 7) or blood cardioplegic solution (group 2, *n* = 7) delivered at 50 mm Hg until arrest and then at 40 mm Hg (Table I). A 2-minute dose of cold cardioplegic solution was given and topical cooling with iced saline solution was also used. Repeated 2-minute doses of cold cardioplegic solution were given at 40 mm Hg every 20 minutes. After 2 hours of cold cardioplegic arrest with multidose cardioplegia, hearts were reperfused with warm, unmodified blood. After normalization of blood flow 30 minutes after reperfusion, hearts were once again stimulated with bradykinin and then with nitroprusside in a manner identical to that before arrest. Nitric oxide samples were again obtained.

Nitric oxide analysis. After collection, whole-blood samples were immediately placed on ice and then centrifuged at 4° C for 10 minutes at 3200 rpm. The supernatant was removed and frozen at -70° C. Aliquots of 100 μ l were then injected into the measuring apparatus and any nitrite or nitrate was rapidly reduced to nitric oxide by vanadium (III) at 98° C.²⁵⁻²⁷ Oxygen-free purified nitrogen was delivered into a reaction flask and then the nitrogen flowed through the system and was drawn into the Dasibi Chemiluminescence NOx Analyzer (model 2108; Dasibi, Glendale, Calif.) at a constant rate of 200 ml/min with the aid of a vacuum pump. The detector is sensitive to nitrogen dioxide radical, the photon-emitting product generated by the reaction between ozone and nitric oxide. The analyzer was calibrated before each use with a standard mixture of 825 ppb nitric oxide in oxygen-free nitrogen (Scott-Marrin, Inc., Riverside, Calif.). Results were expressed in nanomoles per 100 μ l. The arteriovenous difference was then calculated and multiplied by the coronary blood flow to obtain a release rate of nitric oxide in nanomoles per minute.

Statistics. A paired *t* test was used to compare the change in coronary blood flow with baseline both before and after arrest and reperfusion. A paired *t* test was also used to compare the release of nitric oxide before and after preservation. A *p* value of less than 0.05 was considered significant.

Animal care. All animals were cared for in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Results

Endothelium-dependent vasodilatation. Before cardioplegic arrest, there was a significant increase in coronary blood flow in response to bradykinin infusion in both groups; 245% of baseline in group 1 (1.29 $\pm$ 0.26 versus 2.88 $\pm$ 0.77 ml/gm, *p* = 0.0046) and 265% of baseline in group 2 (1.95 $\pm$ 0.54 versus 4.44 $\pm$ 1.65 ml/gm, *p* = 0.01; Fig. 1). After 2 hours of cardioplegic arrest,

Fig. 1. Endothelium-dependent coronary blood flow. Before cardioplegic arrest, there was a significant increase in coronary blood flow with bradykinin stimulation in both group 1 and group 2, 245% and 265% of baseline, respectively. After UW solution arrest, there was a loss of vasodilatation in response to bradykinin, reaching only 117% of baseline ($p = 0.0011$). Vasodilatory response was preserved in hearts protected with low-potassium blood cardioplegia (group 2). *NS*, Not significant; *Blood CP*, blood cardioplegia.

there was a near-complete loss of the vasodilatory response to bradykinin in hearts protected with UW solution; response reached only 117% of baseline, which was not significantly different from baseline (1.31 ± 0.54 versus 1.51 ± 0.6 ml/gm, $p = 0.1$). There was, however, a significant difference ($p = 0.0011$) between prearrest and postarrest response in group 1 (Fig. 1). In contrast, hearts protected with blood cardioplegic solution retained their ability for bradykinin-induced coronary artery vasodilatation; 222% of baseline after arrest versus 265% of baseline before arrest (Tables II and III).

Endothelium-independent vasodilatation. Before cardioplegic arrest, there was a significant increase in coronary blood flow in response to a 1-minute infusion of sodium nitroprusside; 186% of baseline in group 1 (1.19 ± 0.29 versus 2.23 ± 0.33 ml/gm, $p = 0.0001$) and 222% of baseline in group 2 (1.83 ± 0.71 versus 3.85 ± 0.86 ml/gm, $p = 0.0001$; Fig. 2). After 2 hours of multidose cardioplegia with UW solution, the response to sodium nitroprusside was unchanged from prearrest values, reaching 173% of baseline (1.17 ± 0.43 versus 2 ± 0.71 ml/gm, $p = 0.0008$; Fig. 2). This indicates preservation of the endothelium-independent vasodilatory response. Hearts protected with blood cardioplegic solution demonstrated a small but significant ($p = 0.006$) decrease in vasodilatory response to nitroprusside after 2 hours of cardioplegic arrest, with 176% after arrest versus 222% before arrest (Tables II and IV).

Nitric oxide release. The mean nitric oxide release in response to bradykinin stimulation was 96.2 ± 32.0 nmol/min in hearts in group 1 before arrest (Table III). After 2 hours of arrest with UW solution, there was a complete loss of nitric oxide release in response to brady-

kinin, at -32.7 ± 9 nmol/min ($p = 0.0039$ compared with prearrest values; Fig. 3). This loss of nitric oxide release correlated closely with the lack of vasodilatation observed in response to bradykinin stimulation in this group after UW cardioplegic arrest.

Discussion

Donor organ availability remains the major limitation in cardiac transplantation. For this reason, more efficient use of donor hearts is necessary. Investigations into methods of preservation^{1-5, 24} and reperfusion²⁸⁻³¹ have resulted in improved organ function and lengthened the period of safe ischemia.^{1-3, 28} UW solution has been shown in several laboratory and clinical studies^{4, 5} to result in superior functional recovery of transplanted hearts. UW solution is an intracellular solution that contains a 140 mEq/L concentration of potassium, compared with the 10 to 20 mEq/L in most extracellular crystalloid cardioplegic solutions and in blood cardioplegic solution. UW solution also contains several unique ingredients designed to decrease intracellular and interstitial edema, provide metabolic support, and scavenge oxygen-derived free radicals.

We recently demonstrated in our laboratory the ability of UW solution to protect the immature myocardium when used for multidose cardioplegia.³² In an isolated, blood-perfused working heart model, significantly improved recovery of stroke work index was obtained after 2 hours of cold cardioplegic arrest with UW solution as compared with blood cardioplegic solution. Functional recoveries were 100%, 86%, 79%, and 80% at left atrial pressures of 3, 6, 9, and 12 mm Hg, respectively, in hearts protected with UW solution, compared with 65% to 70%

Table II. Coronary blood flows in milliliters per gram of heart tissue

Heart	Baseline	Bradykinin before	Baseline	Nitroprusside before	Baseline	Bradykinin after	Baseline	Nitroprusside after
<i>Group 1</i>								
1	1.33	2.33	0.83	2.00	0.75	0.83	0.67	1.17
2	1.45	2.55	1.32	2.00	1.18	1.18	0.91	2.09
3	1.69	2.34	1.66	2.62	2.03	1.86	1.66	2.62
4	1.44	3.04	1.44	2.52	1.04	1.44	1.44	2.60
5	1.11	2.22	1.11	2.00	2.11	2.67	1.67	2.67
6	1.06	3.33	1.06	1.89	1.11	1.28	1.11	1.94
7	0.94	4.35	0.94	2.59	0.94	1.29	0.71	0.94
Mean	1.29	2.88	1.19	2.23	1.31	1.51	1.16	2.00
SD	0.24	0.71	0.27	0.30	0.50	0.55	0.40	0.66
<i>Group 2</i>								
1	2.09	4.04	1.83	3.48	2.61	4.52	2.52	3.30
2	1.79	6.13	1.70	4.06	1.79	2.74	1.60	2.17
3	3.05	4.69	3.20	5.08	3.05	4.77	3.05	3.98
4	1.89	4.39	1.89	4.62	1.89	3.56	1.59	3.41
5	1.90	5.50	1.90	3.70	2.50	6.30	2.70	4.20
6	1.43	4.11	1.43	3.57	1.43	5.63	1.52	3.57
7	1.48	5.22	0.87	2.43	2.61	6.26	2.26	4.87
Mean	1.95	4.87	1.83	3.85	2.27	4.82	2.18	3.64
SD	0.50	0.72	0.65	0.79	0.53	1.25	0.57	0.78

New baseline flows were measured before each intervention. *SD*, Standard deviation.

Table III. Vasodilatory response and nitric oxide release after a 1-minute infusion of bradykinin (10^{-6} mol/L) before and after cardioplegic arrest

Heart	% baseline CBF		NO release (nmol/min)	
	Pearrest	Postarrest	Pearrest	Postarrest
<i>Group 1 (UW cardioplegia)</i>				
1	175	111	84	-34
2	165	100	34	-27
3	185	139	-38	-42
4	211	91	195	12
5	200	125	150	-69
6	316	118	148	-22
7	463	137	98	-44
Mean $\pm$ SEM	245 $\pm$ 41	117 $\pm$ 7	96 $\pm$ 30	-32 $\pm$ 9
<i>Group 2 (Blood cardioplegia)</i>				
1	193	173		
2	34	152		
3	154	156		
4	234	188		
5	289	252		
6	288	394		
7	353	240		
Mean $\pm$ SEM	265 $\pm$ 28	222 $\pm$ 32		

Nitric oxide release was not measured in the blood cardioplegia group. *CBF*, Coronary blood flow; *NO*, nitric oxide; *SEM*, standard error of the mean.

in hearts protected with blood cardioplegia. The addition of calcium to UW solution resulted in 100% functional recovery at left atrial pressures of 3, 6, and 9 mm Hg, and 93% recovery at a left atrial pressure of 12 mm Hg. The functional recovery obtained with UW solution or UW solution with added calcium was significantly greater

than that obtained with conventional blood cardioplegia.³²

Despite UW solution's impressive performance, both clinically in transplantation and experimentally for neonatal myocardial protection, increased understanding of the importance of coronary endothelial function and rec-

Fig. 2. Endothelium-independent coronary blood flow. There was no significant difference in vasodilatory response to nitroprusside before and after arrest in hearts protected with UW solution (group 1). There was a small but significant decrease in vasodilatation in hearts protected with blood cardioplegia (group 2). *NS*, Not significant; *Blood CP*, blood cardioplegia.

Fig. 3. Nitric oxide release before and after cardioplegic arrest with UW solution. There was a significant increase in nitric oxide release in response to bradykinin infusion before arrest in group 1. After arrest with UW solution, there was complete loss of nitric oxide release ($p = 0.0039$), which correlated with observed lack of vasodilatation.

ognition that high potassium concentrations may be harmful to endothelium have raised concerns regarding the safety of UW solution.^{19-22, 23} Mankad, Chester, and Yacoub²² have previously showed that preservation with cardioplegic solutions containing a potassium concentration as low as 30 mEq/L can abolish the endothelium-dependent vasodilatory response to 5-hydroxytryptophan. When the potassium concentration was reduced to 20 mEq/L, the 5-hydroxytryptophan-induced endothelium-dependent vasodilatory response was maintained.

Saldanha and Hearse²¹ demonstrated loss of coronary vasodilatation in response to 5-hydroxytryptophan in rat hearts after a 30-minute continuous infusion of cardioplegic solution containing a potassium concentration of 25

mEq/L. Other investigators^{18-20, 33, 34} have also shown functional endothelial injury and platelet deposition after infusion of hyperkalemic cardioplegic solution.

Cartier and colleagues²³ did early work looking specifically at the effect of UW solution on endothelial function, in which either perfusion with or perfusion and storage with UW solution resulted in loss of endothelium-dependent vasodilatation. Nitric oxide was not measured in that study, however. In this study, we clearly demonstrated significant impairment in endothelium-dependent vasodilatation, which is attributable to failure of nitric oxide release by the endothelium after infusion of UW cardioplegic solution.

Mankad, Slavik, and Yacoub³⁵ recently concluded

Table IV. Vasodilatory response to 1-minute infusion of sodium nitroprusside (10^{-6} mol/L) before and after cardioplegic arrest as a percentage of baseline coronary blood flow

Heart	Prearrest	Postarrest
<i>Group 1 (UW cardioplegia)</i>		
1	145	175
2	152	230
3	179	159
4	175	181
5	170	160
6	225	175
7	260	134
Mean $\pm$ SEM	186 $\pm$ 16	173 $\pm$ 11
<i>Group 2 (Blood cardioplegia)</i>		
1	190	131
2	238	135
3	158	131
4	244	214
5	195	156
6	250	236
7	282	216
Mean $\pm$ SEM	222 $\pm$ 16	174 $\pm$ 18

SEM, Standard error of the mean.

that UW solution results in endothelial dysfunction when infused at 15° C but not when infused at 4° C or 10° C. Dysfunction was determined by both impaired vasodilatory response to 5-hydroxytryptamine and increased basal coronary artery resistance.³⁵ Although impaired release of endothelium-derived relaxing factor has been postulated as the cause of impaired vasodilatation after high-potassium cardioplegia, nitric oxide release has not been measured in previous studies.

Cullen, Haworth, and Warren³⁴ attempted to measure in vitro changes in levels of endothelium-derived relaxing factor in response to incubation of pulmonary endothelial cells in UW solution. Surprisingly, release of endothelium-derived relaxing factor in response to bradykinin was maintained in cells incubated with UW solution ($K = 140$ mEq/L) or other crystalloid solutions with potassium concentrations of 80 to 107 mEq/L but was impaired after incubation with a blood cardioplegic solution with a potassium concentration of only 6.8 mEq/L. Cullen, Haworth, and Warren³⁴ attributed the loss in release of endothelium-derived relaxing factor in the blood cardioplegia-incubated cells to the acidity of the solution and not to the potassium concentration. The discrepancy between these findings and those of ourselves and others may be related to the use of isolated cells rather than an intact model, the use of pulmonary rather than coronary endothelial cells, and the basic pH (7.6) of the blood cardioplegic solution used in our investigation.

The duration of endothelial dysfunction after high-potassium cardioplegia is unknown, but certainly this duration influences the importance of our findings. Vanhoute has found that 4 weeks after endothelial injury regenerated endothelium, although structurally normal, has a blunted response to vasodilatory stimuli.¹⁶ Others have demonstrated a hypercontractile state and loss of vasodilatory response even 8 weeks after endothelial injury. Cartier and colleagues³⁶ have shown incomplete recovery of endothelium-dependent vasodilatation and an increased vasoconstrictive potential 8 weeks after endothelial injury, despite the presence of histologically intact endothelial cells.

The significance of impaired endothelium-dependent vasodilatation is uncertain. The endothelium's increased predisposition toward vasospasm and inability to release nitric oxide results in platelet deposition³⁷⁻³⁹ and an increased propensity toward thrombosis. Treatment with arachidonic acid inhibitors such as indomethacin or with calcium-channel blockers has not been effective in modifying the early loss of vasodilatory reserve.⁴⁰

The importance of nitric oxide release in regulation of basal coronary tone has been demonstrated in several investigations.^{11, 41, 42} Endothelium-dependent vasodilatation has been shown to affect regional distribution of myocardial perfusion, specifically increasing subendocardial perfusion and increasing the subendocardial/subepicardial blood flow ratio.⁴¹ Impaired autoregulation may affect the response to certain hemodynamic stresses such as pressure overload, where failure to increase subendocardial blood flow could lead to ischemia. Mankad, Slavik, and Yacoub³⁵ demonstrated basal increase in coronary vascular resistance after infusion of UW solution at 15° C but not at 4° C or 10° C. Loss of endothelium-dependent vasodilatation has been correlated with atherosclerosis.^{16, 43} This may be important with regard to the development of accelerated graft atherosclerosis in the transplanted heart, or to the early onset or progression of coronary artery disease if UW solution is used for myocardial protection in nontransplant cardiac operations.

Summary

In this study and in others,^{17, 19-23} it has been demonstrated that high-potassium cardioplegic solutions such as UW solution result in functional impairment of coronary endothelium. After multidose cardioplegia with UW solution, there is a failure of endothelium-dependent vasodilatation. This loss of vasodilatory reserve corresponds with failure of nitric oxide release by the endothelium. However, the duration of this functional deficit and both the short- and long-term effects are unknown. Fur-

ther studies are necessary to determine the significance of these findings and to evaluate the long-term effects of UW solution on the coronary endothelium.

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