PRESERVATION OF ENDOTHELIUM-DEPENDENT VASODILATION WITH LOW-POTASSIUM UNIVERSITY OF WISCONSIN SOLUTION

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University of Wisconsin solution has provided excellent myocardial preservation. However, the high potassium content of the currently available University of Wisconsin solution has been implicated in coronary artery endothelial damage. We placed 16 neonatal (age 1 to 3 days) Duroc piglet hearts on an isolated nonworking perfusion circuit. Endothelium-dependent and endothelium-independent vasodilation were tested by measuring coronary blood flow after intracoronary infusion of bradykinin (10^{-6} mol/L) and nitroprusside (10^{-6} mol/L), respectively. In addition, nitric oxide levels were measured after bradykinin infusion. The hearts were then arrested blindly with either a modified University of Wisconsin solution (group 1; $n = 8$, $K^+ = 25$ mEq/L) or standard University of Wisconsin solution (group 2; $n = 8$, $K^+ = 129$ mEq/L) by infusion of cardioplegic solution every 20 minutes for a total of 2 hours. After bradykinin infusion, the mean coronary blood flow increased by $237.1\% \pm 14.0\%$ of baseline values before arrest and by $232.8\% \pm 16.0\%$ after arrest in group 1 ($p =$ not significant). As in the first group, the mean coronary blood flow in group 2 increased by $231.1\% \pm 13.7\%$ before arrest; however, the increase in mean coronary blood flow after arrest was significantly attenuated ($163.3\% \pm 12.8\%$, $p < 0.01$). The loss of endothelium-dependent coronary blood flow response in group 2 correlated with a decreased capacity to release nitric oxide after arrest (prearrest 8.25 ± 2.30 nmol/min per gram versus postarrest -2.46 ± 2.29 nmol/min per gram, $p < 0.01$). Endothelium-independent vasodilatory response revealed no significant difference between groups before and after arrest. These results suggest that the low-potassium University of Wisconsin solution provides superior protection of the endothelium by preserving the endothelium-dependent vasodilatory response to nitric oxide release. (J Thorac Cardiovasc Surg 1996;112:103-10)

The University of Wisconsin solution (UWS), developed by Wahlberg, Southard, and Belzer¹ in 1986, has been used successfully to preserve the pancreas,² kidney,³ and liver⁴ in both laboratory and clinical settings. Numerous studies have demonstrated that UWS also provides excellent myocardial preservation in a variety of animal models.⁵⁻⁸ Fur-

thermore, clinical trials at various centers have proved that UWS is a safe and effective preservation solution for human cardiac transplantation.^{9,10} These studies have demonstrated favorable effects of UWS on the myocyte but have not focused on its effects on either the conduction tissues or endothelium. Moreover, the optimum concentration and composition of its components have not been standardized. The high potassium content (129 mmol/L) of UWS has been suspected of damaging the endothelium during preservation.

Potassium is a recognized vascular irritant, and it has been shown to cause marked arterial endothelial damage.¹¹ Carpentier, Murawsky, and Carpentier¹² have demonstrated the endothelial cytotoxicity of hyperkalemic crystalloid cardioplegic solutions to endothelial cells in tissue cultures. We recently

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presented our clinical results that demonstrated twice the prevalence of accelerated graft atherosclerosis at 24 months after transplantation in the patient group that received UWS (21%, $n = 100$) compared with the Stanford solution group (10.5%, $n = 100$). Although UWS contributes significantly to successful myocardial functional recovery after storage, numerous laboratory studies have shown that UWS and other hyperkalemic cardioplegic solutions fail to preserve endothelial function.¹³⁻¹⁶

The endothelium plays a significant role in vaso-regulation and coagulation homeostasis; it produces prostacyclin, plasminogen activator, antithrombin III, and endothelium-dependent relaxation factor (EDRF).^{17, 18} EDRF, which has been demonstrated to be nitric oxide (NO),¹⁹ has major effects on local vasoregulation by modulating vascular tone and inhibiting platelet aggregation and adhesion.^{20, 21} Endothelial damage during preservation exposes collagen fibers and the basement membrane and also induces the release of procoagulants.²² Therefore disruption of the endothelium, and hence its normal physiologic functions, may have deleterious effects on myocardial perfusion, vascular permeability, endothelium-platelet interactions, and, consequently, the long-term outcome after cardiac transplantations.

We hypothesized that by decreasing the potassium content of the UWS we might improve preservation of endothelial function. A previous study in our laboratory has shown that a modified low-potassium (25 mmol/L) UWS and the standard high-potassium UWS provide equivalent myocardial functional preservation.²³ The purpose of this study, therefore, was to examine and compare the effects of the potassium level on endothelial function by preserving hearts with either the standard high-potassium or the modified low-potassium UWS.

Material and methods

Experimental preparation. Hearts were harvested from 12 neonatal (1 to 3 days old) piglets (Duroc) without intervening ischemia and placed on an isolated, nonworking, blood-perfused circuit (Fig. 1). Adolescent Duroc pigs (50 to 70 kg), used as cross circulation support, were anesthetized with intramuscular ketamine (100 mg/kg) and acepromazine (0.1 mg/kg). Continuous intravenous pentobarbital diluted in 5% dextrose (5 mg/ml) and supplemented with boluses of pentobarbital through the femoral vein was administered for maintenance anesthesia. Mechanical ventilation (Bennet MA-1 ventilator, Puritan-Bennet Corp., Los Angeles, Calif.) at an inspired oxygen fraction of 1.0, a positive end-expiratory pressure of 5 mm Hg, a tidal volume of 15 to 20 ml/kg, and a rate

of 12 breaths/min was established. Arterial blood gas values were obtained every 30 minutes (model ABL-330, Radiometer, Copenhagen, Denmark) and hematocrit values every hour. The pH was maintained between 7.40 and 7.50, carbon dioxide tension between 30 and 40 mm Hg, and oxygen tension higher than 400 mm Hg. Rectal temperature was maintained at 38° C by use of a heated water blanket (Medi-therm, model No. MTA-4700, Gaymar Industries, Inc., Orchard Park, N.Y.). Systolic blood pressure was kept greater than 80 mm Hg by intravenous lactated Ringer's standard solution or 6% hetastarch and the hematocrit level was kept greater than 25% by providing intravenous blood obtained from a previous support pig.

Blood from the support animal was directed through a roller pump (model 10-40-00, Stokert Instruments, Munich, Germany), warmed to 38° C by a blood cardioplegia device (model BCD ADV-1, Shiley Inc., Irvine, Calif.), and delivered through the innominate artery to the aortic root of the harvested heart at a constant mean pressure of 60 mm Hg. Perfusion and suspension of the heart were achieved by connection of the innominate artery cannula to the blood cardioplegia device while the distal aorta remained clamped. Concurrently, the aorta was cannulated with a 14-gauge perfusion cannula and connected to an open adjustable column to control the coronary perfusion pressure. Silicone rubber tubing (1/4-inch outer diameter) was inserted into the pulmonary artery to collect coronary sinus effluent and measure coronary blood flow (CBF). Blood from the aortic column and pulmonary artery was returned to the support animal directed through a 40 μ m blood filter (Ultipor EC3840, Pall BioMedical Products Corp., East Hills, N.Y.).

Pressure monitoring. Fiberoptic transducer-tipped catheter (1.3 mm outer diameter, model 110-4, Camino Laboratories, San Diego, Calif.) was advanced into the left ventricle via the left atrium for pressure monitoring. Aortic root pressure was simultaneously recorded with a fluid-filled transducer (Statham P23, Spectramed Inc., Oxnard, Calif.).

Endothelial function test. After stabilization of baseline CBF (30 to 60 minutes after perfusion), endothelium-dependent vasodilation was tested by administration of bradykinin mixed in arterial blood to a final concentration of 10^{-6} mol/L for 1 minute at a constant mean pressure of 60 mm Hg. Immediately after the infusion, three separate minute volumes of coronary effluent were collected in graduated cylinders, and the average CBF was calculated and expressed as a percent increase in baseline CBF. In addition, 5 ml samples of arterial and coronary sinus blood were obtained immediately after the 1-minute bradykinin infusion for NO level determination.

After return of CBF to a steady baseline level (approximately 30 minutes after bradykinin infusion), endothelium-independent relaxation was tested by infusing sodium nitroprusside mixed in blood to a final concentration of 10^{-6} mol/L in the same manner as that of bradykinin, and the average CBF per minute was expressed as percent increase of baseline CBF before nitroprusside infusion.

The hearts were then arrested with either the modified low-potassium UWS (group 1, $n = 8$, $K^+ = 25$ mEq/L) or the standard high-potassium UWS (group 2, $n = 8$, $K^+ =$

Fig. 1. Diagram of isolated nonworking perfusion circuit. BCD, Blood cardioplegia device; A, artery; V, vein; Ao, aorta; RA, right atrium; LA, left atrium; PA, pulmonary artery; RV, right ventricle; LV, left ventricle.

129 mEq/L) delivered at 50 mm Hg until arrest and then at 40 mm Hg for a period of 2 minutes while topical cooling was provided with iced saline slush. Two-minute doses of cold cardioplegic solution were given at 40 mm Hg every 20 minutes. After 2 hours of arrest, the hearts were reperfused with warm (37° C) unmodified blood at a constant mean pressure of 40 mm Hg, which was raised to 60 mm Hg after the onset of sinus rhythm. After stabilization of baseline CBF (approximately 1 hour after reperfusion), the prearrest experimental protocol with bradykinin and nitroprusside infusion was repeated. NO samples were obtained after the bradykinin infusion.

NO 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 obtained and kept at 4° C. Aliquots of 100 μ l were then injected into the measuring apparatus and any nitrite or nitrate was rapidly reduced to NO by vanadium at 98° C.^{19, 24} Oxygen-free purified nitrogen was delivered into a reaction flask and was drawn into the Dasibi Chemiluminescence NO_x 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 the nitrogen dioxide radical, the photon-emitting product

generated by the reaction between ozone and NO. The analyzer was calibrated before each use with a standard mixture of 825 ppm NO 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 CBF to obtain the release rate of NO in nanomoles per minute.

Statistical analysis. A two-tailed, paired *t* test was used to compare the percentage increase in CBF from the baseline value before and after arrest and to compare the release of NO before and after preservation. A *p* value of less than 0.05 was considered to be statistically 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 vasodilation. The percentage increase of CBF in response to bradykinin

Fig. 2. Endothelium-dependent vasodilation after bradykinin infusion.

was significant in both groups before arrest: $237\% \pm 14.0\%$ of baseline in group 1 ($n = 8$, 15 ± 1.81 versus 35.02 ± 4.13 ml/min, $p = 0.001$) and $231\% \pm 13.7\%$ of baseline in group 2 ($n = 8$, 14.89 ± 1.03 versus 31.94 ± 3.47 ml/min, $p = 0.001$). There was no significant difference in vasodilatory response between the two groups ($p = 0.58$). After 2 hours of multidose cardioplegic arrest, the percent increase of CBF in response to bradykinin was maintained in group 1, in contrast to the result in group 2: the percent increase was $232\% \pm 16.0\%$ of baseline in group 1 ($n = 8$, 22.88 ± 1.72 versus 53.06 ± 5.14 ml/min) but only $163\% \pm 12.8\%$ of baseline in group 2 ($n = 8$, 24.5 ± 1.66 versus 36.38 ± 1.74 ml/min; Fig. 2). There was no significant difference between prearrest and postarrest responsiveness in group 1 ($p = 0.59$); however, the vasodilatory response to bradykinin was significantly decreased after arrest compared with the prearrest response in group 2 ($p = 0.002$) (Fig. 2).

NO release. The mean NO release after stimulation with bradykinin revealed no significant difference between prearrest (8.63 ± 2.91 nmol/min per gram) and postarrest values (8.06 ± 2.51 nmol/min per gram; $p = 0.88$) in group 1 (Fig. 3). In contrast, there was a significant decrease in NO release in the hearts preserved in high-potassium UWS (group 2) after arrest compared with the prearrest level (pre-arrest 8.25 ± 2.3 versus postarrest -2.46 ± 2.29 nmol/min per gram, $p = 0.005$; Fig. 3).

Endothelium-independent vasodilation. Before cardioplegic arrest, a significant increase of CBF was observed in both groups: $194\% \pm 7.5\%$ of baseline value in group 1 (14.63 ± 1.87 versus 27.96 ± 3.20

Fig. 3. NO release after bradykinin infusion.

ml/min, $p = 0.001$) and $209\% \pm 13\%$ of baseline value in group 2 (14.06 ± 1.30 versus 30.19 ± 4.20 ml/min, $p = 0.001$) (Fig. 4). After 2 hours of arrest, the response to nitroprusside remained unchanged in both groups: $187\% \pm 10\%$ of baseline value in group 1 (20.0 ± 2.49 versus 36.88 ± 3.86 ml/min, $p = 0.001$) and $194\% \pm 17\%$ of baseline value in group 2 (16.75 ± 2.27 versus 30.27 ± 2.36 ml/min, $p = 0.001$) (Fig. 4). Endothelium-independent vasodilatory response to nitroprusside revealed no significant difference between groups before and after arrest ($p = 0.33$ for group 1 and $p = 0.75$ for group 2; Fig. 4).

Discussion

Our previous investigations have revealed that multidose UWS containing 129 mmol/L of potassium is detrimental to endothelial function,²⁴ although UWS is generally known to be clinically and experimentally successful in extended solid organ preservation (kidney, liver, pancreas).²⁻⁴ Studies from numerous laboratories have demonstrated superior functional recovery and ischemic periods of up to 24 hours with the use of UWS.^{7,8} UWS is a formula designed for the induction and safe maintenance of organ preservation for transplantation. Its components are designed to minimize the harmful effects of hypothermic ischemia and subsequent reperfusion. Osmotically active impermeants, that is, lactobionate, raffinose, and pentastarch, have been incorporated to improve myocardial recovery by reducing edema during hypothermic arrest. Other components include allopurinol to reduce oxygen free radical production, glutathione to act as an antioxidant, and adenosine to supply the cell with adenosine triphosphate substrates during reperfusion.²⁵

Nevertheless, the high potassium concentration of UWS has been implicated in endothelial cell damage, causing vasospasm and increased capillary permeability.²⁶ Sellke and colleagues²⁷ have shown that the higher concentration of potassium predisposes the myocardial cell to the influx of calcium, thereby increasing wall tension. As the concentration of potassium increases from 30 to 40 mmol/L, the resting membrane potential partially depolarizes, the calcium channel is activated, and influx increases, which results in an energy-consuming semisystolic arrest. Furthermore, the low sodium concentration of UWS (30 mmol/L) may exacerbate the calcium influx, especially during reperfusion.²⁷ Mankad, Chester, and Yacoub¹³ reported that rat hearts perfused with cardioplegic solution containing a potassium concentration of 30 mEq/L or greater showed decreased endothelial response to vasodilators. Saldanha and Hearse¹⁴ reported endothelial dysfunction in response to 5-hydroxytryptamine in the rat heart after a 30-minute continuous infusion of 20° C cardioplegic solution containing 25 mmol/L of potassium. He and associates,^{27a} however, reported that endothelium of porcine epicardial coronary arteries is relatively tolerant to hyperkalemia-induced injury at a potassium concentration of 50 mmol/L. In contrast, our low-potassium group (25 mmol/L) demonstrated that bradykinin-induced endothelium-dependent vasodilatory function is maintained.

The foregoing discrepancy in the literature suggests that other factors, including oxygen level, ischemia or reperfusion, composition and temperature of preservatives and perfusates, and reperfusion pressure, may also influence endothelial function. Ischemia alone can cause endothelial damage, perhaps mediated by oxygen-derived free radicals, resulting in the impairment of endothelium-dependent vasorelaxation caused by EDRF/NO agonists.^{28, 29} Reperfusion with unmodified blood impairs endothelial function in coronary arteries subjected to normothermic global ischemia.³⁰ Qui, Manche, and Hearse³¹ demonstrated that, after both ischemia and reperfusion with a low-potassium solution (16 mmol/L), endothelium-mediated vasodilation was attenuated by 50%. Endothelial generation of superoxide radicals may be a trigger mechanism for endothelial dysfunction, which is then amplified by neutrophil adherence and migration into the ischemic region.³²

Several investigators have examined the effect of temperature on endothelial function. Amrani and colleagues³³ demonstrated that moderate hypother-

Fig. 4. Endothelium-independent vasodilation after nitroprusside infusion.

mia (20° C) and extremely hyperkalemic UWS for cardioplegia (130 mmol/L) induces a marked increase in coronary vascular resistance that is associated with impaired myocardial protection and the endothelial function is better preserved at 4° C than at 20° C.³⁴ Aoki, Kawata, and Mayer³⁵ reported excessive cold cardioplegic solution (lower than 4° C) may cause endothelial dysfunction. Mankad, Slavik, and Yacoub³⁶ have shown that the optimal temperature to preserve endothelial function after preservation with UWS is at or below 10° C. Our cardioplegic temperature was maintained at 4° C; this may have contributed to the preservation of endothelial function in the low-potassium group.

Evora, Pearson, and Schaff³⁷ have recently shown that hyperkalemic (45 mmol/L) crystalloid cardioplegia does not impair endothelial function in isolated canine epicardial coronary arteries subjected to 45 minutes of arrest. They concluded that endothelial damage may be induced by other mechanisms, including high shear stress from large delivery volumes and high infusion pressure.³⁷ There is a possibility that high shear stress and pressure to the coronary endothelium during reperfusion impair endothelial function by mechanical dislodgment of weakened endothelial attachments. Sawatari and colleagues³⁸ showed that high initial perfusion pressure (60 mm Hg) reduced coronary vasodilatory response to acetylcholine stimulation. In the present study, reperfusion was initiated at 40 mm Hg and then gradually increased to a mean of 60 mm Hg after resumption of cardiac rhythm.³⁸ However, it is still unclear whether a pressure of 40 mm Hg is low enough to avoid endothelial damage. Further stud-

ies are necessary to determine the effects of pressure and flow on the endothelium.

Punctuating the variability noted in the literature concerning the multitude of factors that affect endothelial function is our previous report,²⁴ which, although corroborating our current findings of endothelial dysfunction with use of conventional UWS, differs in one regard. Whereas our prior study revealed concordant elimination of endothelial response to bradykinin and NO production, our present study noted that the endothelial response was reduced but not eliminated in the setting of absent NO production. It is not clear how these apparent contrasting results can be explained. As discussed in the foregoing paragraphs, many factors influence endothelial function, and some of these factors may have played a hand in our conflicting results. Further study is ongoing to examine the effects of these factors. Nevertheless, it is clear from our studies that endothelial function is adversely affected by higher potassium concentrations in crystalloid cardioplegic solution.

It is interesting to note in addition that, although we found an ongoing but diminished endothelial response to bradykinin stimulation, NO production ceased by our current methods of measurement. Two possibilities exist to account for this discordance. First, bradykinin has known mechanisms of vasodilation that, although endothelium dependent, are independent of NO production. One such mechanism is bradykinin-induced generation of prostacyclin, a known vasodilator.³⁹ The prostacyclin pathway may have a differential sensitivity to the effects of potassium cardioplegia than NO. A second and alternative explanation is that current methods for measurement of NO may not be sufficiently sensitive to detect the small amounts of NO release capable of inducing vasodilation. It is likely that both these factors are operative in our present study.

It is now apparent that the vascular endothelium is an active organ producing at least several substances that regulate vascular tone. Furchgott and Zawadzki⁴⁰ found a potent endothelium-derived vasodilator that has been subsequently named EDRF. Extensive evidence exists that EDRF is NO itself. However, there are several important differences between these compounds, specifically their chemical characteristics, half-lives, sensitivities, and sites of action.^{19, 41, 42} The principal biochemical mechanism of action of EDRF/NO has been elucidated. Subsequent to its release, NO, a highly reactive simple gas gener-

ated from the vascular endothelium, penetrates adjacent smooth muscle cell membranes and binds to the heme portion of guanylate cyclase. Activated guanosine cyclase increases the intracellular cyclic guanosine monophosphate level, which in turn reduces the intracellular calcium concentration.⁴³ Sellke and associates⁴⁴ showed that bradykinin induces endothelium-dependent relaxation, which is mediated by EDRF, by demonstrating that selective removal of coronary endothelium or depletion of vascular smooth muscle cyclic guanosine monophosphate markedly reduces the relaxation response to bradykinin stimulation in porcine microvessels. Linz, Martorana, and Schlekens⁴⁵ reported that bradykinin in concentrations as low as 10^{-9} mmol/L increases CBF in a concentration-dependent manner in isolated working perfused hearts with regional myocardial ischemia. We used a bradykinin concentration of 10^{-6} mmol/L, which resulted in an increase in CBF by greater than 230% before arrest. This responsiveness was maintained after preservation with a potassium concentration of 25 mmol/L, but not in high-potassium UWS. This loss of endothelium-dependent relaxation correlated with decreased NO release. Hence it appears that endothelial dysfunction is secondary to a high potassium concentration.

Optimal myocardial preservation remains a major concern in heart transplantation. Fullerton and associates⁴⁶ demonstrated coronary vasomotor dysfunction and histologic evidence of coronary vascular damage acutely after cardiac autotransplantation, which may compromise myocardial function. Furthermore, in the long term, this could contribute to the development of graft coronary artery disease.⁴⁶ Our previously published data showed a significantly lower prevalence of cardiac allograft vasculopathy in hearts preserved with Stanford solution compared with hearts in the UWS group at 24 months of mean follow-up.⁴⁷ Other investigators have correlated endothelial dysfunction with graft atherosclerosis.^{48, 49} This might be important in the progression of accelerated graft atherosclerosis in human heart transplantation. Further investigation is being done to evaluate the long-term effects of endothelial dysfunction, induced by high-potassium UWS, on the development of accelerated graft coronary artery disease.

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