Blood cardioplegia supplementation with the sodium-hydrogen ion exchange inhibitor cariporide to attenuate infarct size and coronary artery endothelial dysfunction after severe regional ischemia in a canine model

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Background: Activation of the sodium-hydrogen ion exchange mechanism results in accumulation of intracellular calcium through the sodium-calcium ion antiport mechanism. Administration of a sodium-hydrogen ion exchange inhibitor before or during ischemia attenuates myocardial ischemia and reperfusion injury. However, the cardioprotection exerted by sodium-hydrogen ion exchange inhibitors as adjuncts to cardioplegia without perioperative administration has not been tested in a model of surgical reperfusion of acute coronary occlusion with cardiopulmonary bypass. This study tested the hypothesis that sodium-hydrogen ion exchange inhibitor-supplemented blood cardioplegia would reduce postcardioplegia injury after severe regional ischemia.

Methods: In anesthetized open-chest dogs, the left anterior descending coronary artery was occluded for 75 minutes, after which total cardiopulmonary bypass was initiated. After crossclamping, cold (4°C) antegrade blood cardioplegia was delivered every 20 minutes for a total of 60 minutes of cardioplegic arrest. In 8 dogs, the blood cardioplegic solution was unsupplemented (vehicle group), whereas in 8 others the solution was supplemented with the sodium-hydrogen ion exchange inhibitor cariporide (10 $\mu\text{mol/L}$, cariporide group).

Results: In the in vitro studies, the direct effects of cariporide on neutrophil function were determined. Isolated canine neutrophils were stimulated by platelet activating factor. Cariporide attenuated superoxide anion production in a concentration-dependent manner, with no appreciable effect at 10 $\mu\text{mol/L}$ (the concentration used in blood cardioplegia) and a peak effect at 100 $\mu\text{mol/L}$. In the in vivo cardiopulmonary bypass model, infarct size was significantly ($P < .05$) smaller in the cariporide group than in the vehicle group ($22.4\% \pm 3.5\%$ vs $40.1\% \pm 5.1\%$ of area at risk), although there were no group differences in postischemic regional wall motion after 2 hours of reperfusion ($0.1\% \pm 0.9\%$ vs $-0.2\% \pm 0.3\%$ systolic shortening). Transmural myocardial edema in the area at risk was significantly decreased in the cariporide group ($80.6\% \pm 0.5\%$) relative to the vehicle group ($83.1\% \pm 0.6\%$). Myeloperoxidase activity in the area at risk, an index of neutrophil accumulation,

was significantly lower in the cariporide group than in the vehicle group (4.7 ± 0.9 absorbance units/[min · g tissue] vs 10.3 ± 2.3 absorbance units/[min · g tissue]). In isolated postischemic left anterior descending coronary artery rings, maximum relaxation in response to the endothelium-dependent vasodilator acetylcholine was significantly greater in the cariporide group than in the vehicle group ($77.5\% \pm 7.4\%$ vs $51.4\% \pm 8.0\%$), whereas smooth muscle relaxation in response to nitroprusside was comparable between groups.

Conclusion: In this canine model, supplementation of blood cardioplegia with cariporide, a sodium-hydrogen ion exchange inhibitor, reduced infarct size, attenuated neutrophil accumulation in the area at risk, and reduced postischemic coronary artery endothelial dysfunction without directly inhibiting neutrophil activity. Cariporide as an adjunct to blood cardioplegia without perioperative administration attenuated surgical ischemia-reperfusion injury in jeopardized myocardium.

Cardiac surgery is performed on hundreds of thousands of patients each year to alleviate coronary artery disease, valve problems, and other cardiac diseases. Although the operative outcomes with current formulations of cardioplegic solutions are generally acceptable, with low morbidity and mortality, patients at high risk, such as those with an evolving myocardial infarction, low ejection fraction, or cardiogenic shock, require additional strategies to adequately protect the vulnerable myocardium during cardiac surgery. Current strategies in on-pump myocardial protection advocate the use of cardioplegic solutions not only to arrest the heart but to act as a vector by which to introduce cardioprotective agents targeting specific mechanisms of ischemia-reperfusion injury.

Activation of the $\text{Na}^+\text{-H}^+$ exchanger (NHE) has been implicated in the pathogenesis of tissue ischemia-reperfusion injury.¹⁻³ Under normal conditions, the $\text{Na}^+\text{-H}^+$ antiporter mechanism operates in the forward direction, driven by the inwardly directed transmembrane sodium ion gradient and expelling a single intracellular hydrogen ion in exchange, thereby maintaining intracellular pH and regulating intracellular sodium level.⁴ During ischemia, however, the accumulation of intracellular hydrogen provides the greater driving force for the antiporter, with a resulting efflux of hydrogen ion to the extracellular compartment and a net intracellular accumulation of sodium. The robust buildup of protons in the extracellular compartment during ischemia eventually reduces the transmembrane gradient, which in turn may inhibit NHE activity and increase sodium accumulation. At reperfusion, however, washout of protons in the extracellular compartment restores a transmembrane gradient favoring proton efflux. Thus the antiporter becomes reactivated during reperfusion,⁵ with a resulting net influx and accumulation of intracellular sodium. The con-

sequent accumulation in intracellular sodium stimulates calcium ion influx through the $\text{Na}^+\text{-Ca}^{2+}$ antiporter and accumulation of intracellular free calcium. Intracellular calcium ion accumulation has been implicated in the pathogenesis of systolic and diastolic dysfunction and in the transition to irreversible tissue injury after ischemia and reperfusion.^{6,7} Accordingly, inhibition of the NHE mechanism has been advocated as a cardioprotective strategy.⁸⁻¹⁰ Previous studies have consistently demonstrated that inhibition of the NHE mechanism results in decreased accumulation of intracellular sodium⁴ and subsequently decreased calcium overload and myocardial damage during nonsurgical ischemia and reperfusion when administered before the ischemic event.^{1,9,11} Indeed, the cardioprotection during coronary occlusion and reperfusion observed with NHE inhibition rivals that observed with ischemic preconditioning, and it may in fact be synergistic with preconditioning or may exceed the cardioprotection observed with preconditioning.¹² However, whether cardioprotection is exerted when the NHE inhibitor is administered at reperfusion remains controversial.¹³⁻¹⁵

In cardiac surgery under cardiopulmonary bypass (CPB) and intermittent cardioplegia, potential injury may occur during intraoperative ischemia between multidose infusions of cardioplegic solution or as a result of maldistribution of solution distal to total coronary occlusions. This intraoperative injury may be additive to the antecedent injury caused by regional ischemia, heart failure, cardiogenic shock, and so on. In addition, there is potential for reperfusion injury during each infusion of cardioplegic solution and after removal of the aortic crossclamp. Neutrophils play an important role in the pathogenesis of ischemia-reperfusion injury,¹⁶ particularly in cardiac surgery, where the extracorporeal circuitry amplifies the inflammatory response.¹⁷

Several studies performed in isolated perfused-heart preparations have indicated that inclusion of amiloride analogs or other NHE inhibitors attenuates postischemic or postcardioplegia contractile dysfunction.^{18,19} Moreover, in a clinical randomized trial (the GUARDIAN trial²⁰) the selective NHE type 1 isoform (NHE-1) inhibitor cariporide (HOE 642) was administered intravenously during the perioperative period to high-risk patients who underwent coronary artery bypass grafting. This study reported a significant reduction in combined infarction and mortality. However, few reports have tested the cardioprotective effects of NHE inhibitors with in vivo models of surgical revascularization in which neutrophils may participate in the inflammatory component of reperfusion injury. Neither have any studies to date tested the potential benefits of NHE inhibitors as an adjunct to blood cardioplegia in vivo. Accordingly, this study tested the hypothesis that the selective NHE inhibitor cariporide would attenuate postcardioplegia injury when given as an additive to blood cardioplegic solution in a canine model simulating on-pump surgical revascularization of evolving myocardial infarction.

Material and Methods

Experimental Preparation

All animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996.

In vitro superoxide anion generation by activated neutrophils.

Experiments were performed to determine the direct effects of cariporide or neutrophil function. To isolate neutrophils, peripheral canine blood (200 mL) was mixed with 45 mL of anticoagulating agents, which included 1.6% citric acid and 2.5% sodium citrate (pH 5.4), and 100 mL of 6% dextran solution in buffered Hanks balanced salt solution. Polymorphonuclear neutrophils were isolated with the Ficoll-Paque (Sigma Chemical, St Louis, Mo) technique described in detail elsewhere.²¹ The cells were adjusted to approximately 9×10^7 cells/mL. Final suspensions contained $94\% \pm 1\%$ neutrophils, and cell viability averaged $99\% \pm 0.5\%$, as determined by trypan blue exclusion. Superoxide anion production by neutrophils stimulated by platelet activating factor (100 nmol) were determined by measuring the superoxide dismutase-inhibitable reduction of ferricytochrome C to ferrocyanochrome C spectrophotometrically at 550 nm with a V-Max Microtiter Plate Reader (Molecular Devices Corporation, Sunnyvale, Calif). In addition, degranulation of platelet-activating factor-stimulated neutrophils was determined by analysis of myeloperoxidase activity.

In vivo study. Twenty healthy mongrel dogs of either sex weighing 22.4 to 31.6 kg (average weight 27.4 ± 1.0 kg) were initially anesthetized by intravenous administration of 2.5% thio-pental (20 mg/kg) followed by constant infusion of fentanyl citrate ($0.4 \mu\text{g}/[\text{kg} \cdot \text{min}]$) and diazepam ($0.003 \text{ mg}/[\text{kg} \cdot \text{min}]$) during the experiment. Each dog was endotracheally intubated and mechan-

ically ventilated to maintain Pao_2 at greater than 100 mm Hg, Paco_2 between 35 and 45 mm Hg, and pH between 7.35 and 7.45. Metabolic acidemia was corrected with sodium bicarbonate infusion as needed. Polyethylene catheters were inserted into the femoral artery for arterial blood sampling and for monitoring systemic arterial blood pressure, and a catheter was inserted into the femoral vein for fluid administration. A rectal temperature probe was inserted to measure core body temperature. After median sternotomy, the superior and inferior venae cavae were loosely encircled with umbilical tapes, the azygos vein was ligated, and the heart was suspended in a pericardial cradle. Millar catheter-tipped pressure transducers (model MPC-500; Millar Instruments, Inc, Houston, Tex) were placed into the proximal aorta through the right internal thoracic artery and into the left ventricular cavity through an apical stab incision. A 1-cm portion of the left anterior descending coronary artery (LAD) distal to the first diagonal branch was dissected and loosely encircled with a 2-0 silk suture, which was later used to produce regional myocardial ischemia. A pair of 5-MHz piezoelectric ultrasonic crystals, 2.5 to 3.0 mm in diameter, was placed in the subendocardium of the myocardium perfused by the LAD (ischemic-reperfused segment) to measure instantaneous segmental dimensions with a sonomicrometer (model 120; Triton Technology, Inc, San Diego, Calif).

The dogs were systemically anticoagulated with sodium heparin at 300 U/kg, supplemented every 90 minutes with an additional 150 U/kg to maintain the activated clotting time at more than 400 seconds. The left subclavian artery was cannulated for aortic perfusion, and the superior and inferior vena caval cannulas were inserted through the right atrium for venous return. The cannula tips were kept in the atrium until the institution of CPB to avoid hemodynamic interference. A double-lumen cardioplegic catheter (Medtronic DLP, Grand Rapids, Mich) was inserted into the ascending aorta proximal to the aortic crossclamp for the simultaneous delivery of blood cardioplegia and measurement of cardioplegic infusion pressure. Venous blood was oxygenated with a membrane oxygenator (COBE Cardiovascular, Inc, Arvada, Colo) and passed through a $50\text{-}\mu\text{m}$ filter and bubble trap before it was returned to the arterial circulation. The rate of oxygenation was adjusted to maintain the systemic Pao_2 at greater than 200 mm Hg and the Paco_2 between 30 and 40 mm Hg. Metabolic acidosis was counteracted with sodium bicarbonate to maintain arterial blood pH between 7.35 and 7.45. The mean arterial blood pressure during CPB was maintained at 70 mm Hg by adjustment of the blood flow through the arterial cannula. A myocardial temperature probe was placed in the anterior wall of the left ventricle.

Experimental Protocol

After all surgical instrument placement, hemodynamic data and blood samples were obtained at baseline. Each dog then received a bolus injection of lidocaine ($1.2 \text{ mg}/\text{kg}$) followed by a continuous infusion ($0.3 \text{ mg}/[\text{kg} \cdot \text{min}]$), after which the LAD was ligated just distal to the first diagonal branch for 75 minutes. After 75 minutes of LAD regional ischemia, the cannulas were advanced and CPB was initiated. The left ventricle was vented by gravity drainage through a transmyocardial cannula, and the systemic blood temperature was reduced to 28°C . The dogs were randomly allocated into groups that received blood cardioplegia supplemented with either saline vehicle (vehicle group, $n = 8$) or $10 \mu\text{mol/L}$ of HOE

642 (cariporide group, $n = 8$) at equivalent volumes of diluent. The aorta was crossclamped, and cardioplegic arrest was induced with multidose, hypothermic, hyperkalemic blood cardioplegic solution (66 parts blood to 1 part crystalloid) of the following composition: 20.0-mEq/L (induction, terminal) or 10.0-mEq/L (intermittent) potassium ion, 1.0 mmol/L calcium ion, and 0.4-mmol/L magnesium ion, at pH 7.4 with tris(hydroxymethyl)aminomethane. The blood cardioplegic solution was delivered with the Myocardial Protection System cardioplegia and perfusion system (Quest Medical, Dallas, Tex). High-potassium blood cardioplegia (20-mEq/L potassium ion, 4°C) was delivered for 3 minutes during induction, and low-potassium blood cardioplegia (approximately 10-mEq/L potassium ion) was delivered for 2 minutes after 20 and 40 minutes of arrest at an aortic root pressure of 50 mm Hg. The LAD ligature was loosened before the second cardioplegic infusion to simulate revascularization of a target vessel. After 60 minutes of cardioplegic arrest, a hot-shot (20-mEq/L potassium ion, 27°C) infusion of blood cardioplegia was delivered for 3 minutes. Systemic blood was rewarmed to 37°C by the end of the hour of cardioplegic arrest, and the aorta was unclamped to initiate reperfusion. After cardiac electromechanical activity appeared, the mean arterial pressure was gradually increased from 50 to 80 mm Hg through a period of 5 minutes. The heart was maintained on total vented bypass for the first 30 minutes of reperfusion (beating empty reperfusion). Subsequently, all hearts were weaned from CPB and reperfusion was continued in the working state for an additional 90 minutes. The experiment was terminated with a bolus of intravenous sodium pentobarbital (100 mg/kg). The heart was immediately excised and placed in cold Krebs-Henseleit solution oxygenated with 95% oxygen and 5% carbon dioxide and with the following composition: 118.1-mmol/L sodium chloride, 4.7-mmol/L potassium chloride, 1.2-mmol/L monobasic potassium phosphate, 1.2-mmol/L magnesium sulfate, 2.5-mmol/L calcium chloride dihydrate, 25.0-mmol/L sodium hydrogen carbonate, and 11.1-mmol/L glucose at pH 7.4.

Data collection and analysis. Hemodynamic and segmental length data were acquired during a 10-second period of respiratory apnea. The data from each channel were digitized and processed by computer algorithms with an interactive videographics program (SPECTRUM; Wake Forest University, Winston-Salem, NC) as described previously elsewhere.²² Percentage segmental shortening and segmental stroke work were determined as described previously elsewhere.²² Measurements were taken before LAD occlusion (baseline), after 75 minutes of LAD occlusion, after 30 minutes of empty beating reperfusion, and every 30 minutes during the 90 minutes of reperfusion in the off-pump working state.

Determination of area at risk and infarct size. After postexperimental excision of the heart, a catheter was inserted into the aortic root. The LAD ligature was retied, and Unisperse blue pigment (Ciba Specialty Chemicals Company, High Point, NC) was injected under a perfusion pressure of 100 mm Hg to stain the normally perfused region blue and thereby demarcate the area at risk. The left ventricle was cut into 4- to 5-mm thick transverse slices. The area at risk was separated from the nonischemic zone and incubated for 15 minutes in 1% triphenyltetrazolium chloride (Sigma, St Louis, Mo) at 37°C to differentiate the necrotic zone

(pale) from the ischemic nonnecrotic zone (red). The area at risk was calculated as the sum of the weights of the nonnecrotic and necrotic tissues within the ischemic zone, divided by the weight of the left ventricle and expressed as a percentage (area at risk percentage). The infarct size was calculated as the weight of necrotic tissue divided by the weight of the area at risk and expressed as a percentage (infarct percentage).

Plasma creatine kinase activity. Arterial blood samples (3 mL) were centrifuged at 2500g and 4°C for 10 minutes. The plasma was drawn off and analyzed spectrophotometrically for creatine kinase (CK) activity and for protein concentration (CK-10 kit; Sigma Diagnostics, St Louis, Mo). Plasma CK activity was expressed in international units per gram of protein.

Determination of myocardial edema. Postexperimental transmural myocardial tissue samples were taken from the nonischemic zone and from the area at risk. Each sample was further subdivided into subepicardial and subendocardial regions, blotted to remove surface moisture, weighed, and desiccated for 48 hours. Percentage myocardial water content was determined as follows: $[(\text{wet weight} - \text{dry weight}) / \text{wet weight}] \times 100\%$.

Cardiac myeloperoxidase activity. Postexperimental transmural myocardial tissue samples were taken from the nonischemic zone and from the nonnecrotic and necrotic areas of the area at risk for spectrophotometric analysis of myeloperoxidase activity as an assessment of neutrophil accumulation in myocardium, as described previously elsewhere.²³ MPO activity was expressed as the change in absorbance units per minute per gram of myocardial tissue.

Postexperimental coronary artery endothelial function. Both the ischemic-reperfused LAD and nonischemic left circumflex coronary artery (LCx) were carefully dissected from the heart, and endothelial function was quantified by relaxation responses to incremental concentrations of the endothelium-specific nitric oxide synthase stimulator acetylcholine in organ chambers, as described previously elsewhere.²⁴ The vascular rings were placed at the optimal point of their length-tension relationship and incubated with 10- $\mu\text{mol/L}$ indomethacin to prevent vascular responses to endogenous prostacyclin. The coronary rings were then contracted with 10 nmol/L of the thromboxane A_2 mimetic agent U46619 (Pharmacia & Upjohn, Bridgewater, NJ). The procedure was repeated for sodium nitroprusside (Sigma) to test endothelium-independent vascular smooth muscle relaxation. Relaxation was expressed as a percentage of U46619-induced constriction. Drug concentrations were expressed as the final concentration in the organ chamber.

Statistical Analysis

The data were analyzed by 1-way or 2-way analysis of variance for repeated measures to identify group, time, and group-time interactions. If significant interactions were found, then further pairwise analysis was performed by post hoc analysis to locate the source of differences. Comparisons between the two groups in the area at risk, infarct size, MPO activity, myocardial edema, and percentage relaxation of postischemic coronary artery rings at each concentration were analyzed with the Student t test. Data are reported as mean $\pm$ SEM.

TABLE 1. The effect of increasing concentrations of cariporide on superoxide anion generation (nMol/5 × 10⁶ PMN) and degranulation (as a percentage decrease from platelet-activating factor–stimulated degranulation) by neutrophils stimulated by 100-nmol/L platelet activating factor

Cariporide (μmol/L)	Superoxide anion	P value	Degranulation (%)	P value
Control	3.22 ± 0.55*	<.0001	-	
0	30.33 ± 4.49		0	
10	26.06 ± 4.52	.4	6.5 ± 1.8†	.001
50	20.60 ± 4.54	.09	12.3 ± 1.7†	.0001
100	13.88 ± 2.79*	.003	28.9 ± 2.2†	.0001
200	7.5 ± 5.4*	.003	64.2 ± 1.2†	.0001

Control neutrophils were not stimulated with platelet-activating factor.

*P < .05 versus platelet activating factor activated neutrophils (0 concentration cariporide).

†P < .05 versus platelet activating factor-stimulated and untreated group (no cariporide).

TABLE 2. Blood cardioplegia variables

	Group	Initial cold infusion	20-min infusion	40-min infusion	Terminal warm infusion
Blood cardioplegia volume (mL)	Vehicle	384 ± 17	254 ± 18	299 ± 24	422 ± 30
	Cariporide	336 ± 22	277 ± 27	280 ± 19	429 ± 36
Myocardial temperature (°C)	Vehicle	17.0 ± 1.7	11.8 ± 1.5	11.0 ± 1.3	23.2 ± 3.6
	Cariporide	15.1 ± 2.6	9.7 ± 2.3	9.1 ± 1.4	21.1 ± 2.5
Hematocrit (%)	Vehicle	21.6 ± 1.6	20.7 ± 1.6	19.1 ± 1.3	19.9 ± 1.5
	Cariporide	21.7 ± 1.7	19.3 ± 1.0	19.6 ± 0.7	18.4 ± 1.0
pH (at 37°C)	Vehicle	7.36 ± 0.03	7.37 ± 0.04	7.46 ± 0.03	7.44 ± 0.03
	Cariporide	7.34 ± 0.01	7.36 ± 0.01	7.43 ± 0.01	7.42 ± 0.01
Potassium ion (mmol/L)	Vehicle	23.6 ± 1.2	13.9 ± 0.3	14.8 ± 0.5	25.4 ± 1.4
	Cariporide	20.5 ± 1.6	13.8 ± 0.4	14.5 ± 0.4	24.3 ± 1.8
Calcium ion (mmol/L)	Vehicle	0.99 ± 0.06	1.04 ± 0.03	1.06 ± 0.04	1.14 ± 0.07
	Cariporide	0.84 ± 0.08	1.03 ± 0.04	1.07 ± 0.04	1.01 ± 0.08
Magnesium ion (mmol/L)	Vehicle	0.36 ± 0.04	0.40 ± 0.00	0.40 ± 0.00	0.43 ± 0.02
	Cariporide	0.36 ± 0.04	0.37 ± 0.02	0.39 ± 0.01	0.37 ± 0.06
Glucose (mg/dL)	Vehicle	139 ± 9	139 ± 10	157 ± 12	162 ± 14
	Cariporide	116 ± 6	136 ± 8	142 ± 8	156 ± 10
Osmolality (mOsm/kg)	Vehicle	294 ± 2	301 ± 2	300 ± 2	296 ± 2
	Cariporide	300 ± 2	302 ± 4	299 ± 1	298 ± 1

There were no significant differences within groups or between groups. There were no differences between groups at any time.

Results

In Vitro Effect of Cariporide on Neutrophil Superoxide Generation and Degranulation

The effects of cariporide on degranulation and superoxide generation in neutrophils stimulated with platelet-activating factor are summarized in Table 1. Platelet-activating factor significantly increased the superoxide anion generation relative to control, unstimulated neutrophils. There was a general trend toward decreased superoxide anion generation with increasing concentrations of cariporide. However, a significant reduction was observed at cariporide concentrations of 100 μmol/L and 200 μmol/L. Likewise, cariporide maximally inhibited degranulation in a concentration-dependent manner, with a significant and maximum effect at 100 μmol/L. on the basis of these results, a cariporide concentration of 10 μmol/L was chosen to target cardioprotection of myocytes, rather than the higher concentrations necessary to inhibit neutrophil function directly. The con-

centration of cariporide used in the cardioplegic solution thus had no direct effect on neutrophil function.

In Vivo Study

Twenty animals were initially entered into the experimental phase of the study. Four were excluded because of intrac-table ventricular fibrillation during the LAD occlusion. The remaining dogs were equally divided into groups that received blood cardioplegia supplemented with either saline vehicle (vehicle, n = 8) or 10-μmol/L HOE 642 (cariporide, n = 8) at equivalent volumes of diluent.

Myocardial temperature and cardioplegia variables. There were no group differences in blood cardioplegia variables within each time point (Table 2). Anterior myocardial temperatures were not statistically different between the two groups during the initial, maintenance (20 minutes and 40 minutes of infusion), or terminal cardioplegia infusions (Table 2).

TABLE 3. Hemodynamic variables during the course of the experiment

Variable	Group	Baseline	Ischemia	Reperfusion		
				60 min	90 min	120 min
Heart rate (beats/min)	Vehicle	103.0 ± 4.6	124.8 ± 4.4	142.2 ± 2.5	138.1 ± 3.0	133.0 ± 2.6
	Cariporide	100.5 ± 8.0	114.1 ± 8.9	126.8 ± 4.9*	129.6 ± 4.3	126.0 ± 6.5
Mean arterial pressure (mm Hg)	Vehicle	82.0 ± 3.4	77.5 ± 3.2	72.1 ± 1.3	64.5 ± 2.2	71.4 ± 3.0
	Cariporide	76.5 ± 1.3	78.8 ± 2.3	72.1 ± 3.1	70.0 ± 1.9	75.4 ± 1.3
Left ventricular end-diastolic pressure (mm Hg)	Vehicle	6.8 ± 0.9	7.9 ± 0.9	8.3 ± 1.2	7.6 ± 1.0	8.3 ± 1.2
	Cariporide	7.2 ± 0.7	8.7 ± 0.6	9.0 ± 0.5	8.6 ± 0.8	8.5 ± 0.7

* $P < .05$ ($P = .01$) versus vehicle within the same time point.

TABLE 4. Regional cardiodynamic data in the area at risk during the experiment

Variable	Group	Baseline	Ischemia	Reperfusion		
				60 min	90 min	120 min
End-diastolic length (mm)	Vehicle	16.7 ± 1.1	19.9 ± 1.2	18.5 ± 0.9	18.2 ± 0.9	18.3 ± 0.9
	Cariporide	16.4 ± 0.4	20.4 ± 0.7	17.2 ± 0.5	17.1 ± 0.5	17.1 ± 0.5
End-systolic length (mm)	Vehicle	14.2 ± 1.0	21.0 ± 1.2	18.6 ± 1.1	18.4 ± 1.0	18.3 ± 1.0
	Cariporide	13.9 ± 0.5	20.9 ± 0.7	16.7 ± 0.5	16.7 ± 0.5	16.7 ± 0.5
Percentage segmental shortening (%)	Vehicle	15.2 ± 1.3	-5.6 ± 1.3	-0.1 ± 1.0	-0.7 ± 0.7	-0.2 ± 0.3
	Cariporide	15.0 ± 1.7	-5.0 ± 0.8	1.8 ± 0.8	0.8 ± 0.8	0.1 ± 0.9
Segmental stroke work (mm Hg · mm)	Vehicle	184.4 ± 32.9	8.0 ± 4.2	15.2 ± 13.2	1.9 ± 9.5	7.9 ± 4.9
	Cariporide	156.6 ± 17.6	6.3 ± 3.2	43.7 ± 7.4	22.8 ± 6.2*	25.3 ± 8.5†

There were no significant differences between groups.

* $P = .09$.

† $P = .10$.

Hemodynamic data. Hemodynamic data during the course of the experiment are shown in Table 3. There were no significant differences between the two groups at each time point in most hemodynamic variables, except that the heart rate in the vehicle group was significantly greater than that in the cariporide group at 60 minutes of reperfusion.

Cardiodynamic function. Both groups exhibited comparable baseline systolic shortening and systolic dysfunction in the area at risk during LAD occlusion. Hearts that received either cariporide-supplemented blood cardioplegia or unsupplemented blood cardioplegia exhibited poor recovery of percentage segmental shortening in the area at risk throughout reperfusion (Table 4). With segmental stroke work, there was a strong tendency toward a greater recovery in the cariporide group at 90 minutes of reperfusion ($P = .09$) and at 120 minutes of reperfusion ($P = .10$). At these time points, the pressure-segment length loops in the cariporide group were displaced to the left of the baseline loops and demonstrated systolic bulging. There was an "opening" of the loop caused by late systolic contraction, so that positive work was performed. However, the degree of segment work performed was inconsistent, as evidenced by the large SEM. Some inconsistency was also observed in the vehicle group at 90 and 120 minutes of reperfusion.

Area at risk and infarct size. The area placed at risk by the LAD coronary artery occlusion, expressed as area at risk percentage (Figure 1), was comparable between the two groups ($31.3\% \pm 4.1\%$ and $35.3\% \pm 4.0\%$ in the vehicle and cariporide groups, respectively). Infarct size, expressed as infarct percentage (Figure 1), was significantly reduced by nearly half in cariporide-treated hearts ($22.4\% \pm 0.04\%$) relative to the vehicle group ($40.1\% \pm 0.05\%$).

Plasma creatine kinase activity. CK activities at baseline and at the end of 75 minutes of ischemia were comparable between the two groups (Figure 2). There was no significant increase in CK activity during LAD occlusion in either group relative to baseline values. Although both groups showed an increase in plasma CK activity during reperfusion, there was a significant reduction in CK activity throughout reperfusion in the cariporide group relative to the vehicle group (Figure 2). The degree of reduction in CK activity in the cariporide group was qualitatively similar to the reduction in infarct size.

Cardiac myeloperoxidase activity. MPO activities in the nonischemic zone were low and comparable between the two groups (Figure 3). In both the ischemic nonnecrotic and necrotic zones, MPO activities were significantly reduced in the cariporide group relative to the vehicle group, which

Figure 1. Area at risk relative to left ventricular mass (AAR/LV) and infarct size as area of necrosis relative to area at risk (An/AAR) in vehicle (Veh, black bars) and cariporide (NHEI, white bars) groups. Asterisk designates *P* value versus vehicle group.

suggests a reduction in neutrophil accumulation in the area at risk with cariporide-supplemented blood cardioplegia.

Myocardial edema. After 2 hours of reperfusion, the myocardium in the vehicle group demonstrated the greatest edema in the area at risk subendocardium, with tissue water content averaging $84.1\% \pm 0.7\%$ (Figure 4). In the cariporide group, there were reductions in myocardial edema in the subepicardial and subendocardial regions of the area at risk relative to the vehicle group.

Postexperimental coronary artery endothelial function. Vasorelaxation responses to acetylcholine and sodium nitroprusside in isolated postexperimental coronary artery rings taken from the ischemic-reperfused LAD and the nonischemic LCx are summarized in Figure 5. Figure 5, A, shows the percentages of relaxation with vasodilators in the vehicle group. The response to acetylcholine, an endothelium-dependent and muscarinic receptor-mediated vasodilator, was significantly ($P = .04$) blunted in the LAD rings (maximum $51.4\% \pm 8.0\%$ relaxation with the highest concentration) relative to the nonoccluded LCx rings (maximum $79.4\% \pm 10.6\%$). In contrast, the responses to acetylcholine in the LAD (maximum $77.5\% \pm 7.3\%$) and LCx (maximum $85.4\% \pm 6.9\%$) rings were comparable in the cariporide group (Figure 5, B), with LCx rings in the two groups relaxing comparably. The endothelium-independent vasodilator sodium nitroprusside caused comparable relaxations in the two groups (Figure 5, A and B), with no significant difference between the LAD and LCx rings. These data indicate that cariporide-supplemented cardioplegia protected endothelial function from ischemia-reperfusion injury.

Discussion

The $\text{Na}^+\text{-H}^+$ exchange mechanism has been implicated in the pathogenesis of ischemia-reperfusion injury in the myo-

Figure 2. Plasma CK activity during time course of experiment in vehicle (Veh, squares) and cariporide (NHEI, circles) groups. Base, Baseline; Isch, ischemia; R30, 30 minutes of reperfusion; R60, 60 minutes of reperfusion; R90, 90 minutes of reperfusion; R120, 120 minutes of reperfusion. Asterisk designates *P* value versus vehicle group.

cardium.^{1,25} Lethal effects are seen when sodium ion influx in exchange for hydrogen ion efflux stimulates calcium ion accumulation through the $\text{Na}^+\text{-Ca}^{2+}$ antiporter. Accordingly, NHE inhibitors have been advocated as a cardioprotective strategy during nonsurgical myocardial ischemia and reperfusion.^{4,8,10} Previous studies of ischemia-reperfusion have reported significant reductions in infarct size,^{14,15,26,27} reduced incidence of arrhythmias,^{26,28,29} and attenuation of apoptotic cell death^{29,30} with NHE inhibitors. In contrast, the cardioprotective effects afforded by NHE inhibitors during surgical ischemia and reperfusion have to date been examined primarily in isolated perfused-heart studies that used crystalloid cardioplegic solutions, with systolic function as the major end point.^{18,31} In this study we investigated the potential of the NHE inhibitor cariporide (HOE 642) as an adjunct to blood cardioplegia only to reduce postcardioplegia injury after normothermic regional myocardial ischemia. The model of antecedent ischemic myocardium created by coronary artery occlusion was chosen not only to simulate surgical revascularization of acutely ischemic myocardium but also as a model of regionally jeopardized myocardium vulnerable to ischemic and reperfusion injuries. In this model, cariporide in blood cardioplegia used at a concentration of $10\text{ }\mu\text{mol/L}$ did not directly inhibit neutrophil activities but did reduce postischemic infarct size, neutrophil accumulation, and tissue edema in the area at risk. It also reduced coronary artery endothelial dysfunction. This concentration of cariporide is higher than that used in previous studies with isolated crystalloid-perfused hearts (approximately $1\text{ }\mu\text{mol/L}$).

Treatment with cariporide-enhanced blood cardioplegia

Figure 3. MPO activity in nonischemic (*Non-isc*), nonnecrotic (*Non-Nec*), and necrotic area at risk (*AAR*) postexperimental myocardial tissue samples from vehicle (*Veh*, black bars) and cariporide (*NHEI*, white bars) groups. Asterisk designates *P* value versus vehicle group.

Figure 4. Myocardial water content after desiccation of subendocardial (*Endo*) and subepicardial (*Epi*) tissue samples in both area at risk (*AAR*) and nonischemic (*Non-isc*) zones at end of experiment in vehicle (*Veh*, black bars) and cariporide (*NHEI*, white bars) groups. Asterisk designates *P* value versus vehicle group within each area of myocardium.

did not, however, improve functional recovery in the area at risk. Although NHE inhibitors have been reported to be more effective when hearts are treated before the onset of ischemia (coronary artery occlusion) than after ischemia (at reperfusion),^{14,15,32,33} this study clearly demonstrated that NHE inhibitor administration in hypothermic cardioplegia was cardioprotective, in agreement with other studies of crystalloid¹⁸ or blood³⁴ cardioplegic solutions. Previous studies have shown that the NHE mechanism remains functional even under hypothermic conditions³¹ and is therefore a potential target for inhibition when hypothermic condi-

tions are used. Although cariporide attenuates neutrophil activities at higher concentrations, consistent with reports for other NHE inhibitors,³⁵ the concentration used in this study was below that which would directly inhibit neutrophil functions. Thus the cardioprotection was likely exerted on the myocardium directly rather than by antineutrophil mechanisms. In addition, there was no evidence that cariporide may have had a direct protective effect on the coronary artery endothelium. Attenuation of postischemic endothelial dysfunction may also have resulted from a reduction in ischemic injury and a subsequent inflammatory response to reperfusion involving the endothelium.

In the surgical setting used in this protocol, cariporide had no opportunity to intervene during the period of coronary occlusion. However, cariporide could intervene during the ischemia imposed between infusions of cardioplegic solution and could also reduce reperfusion injury. The delivery of blood cardioplegia to ischemic myocardium is, in fact, a form of reperfusion. Maddaford and Pierce²⁵ reported that the NHE mechanism was most active during the initial phase of reperfusion, and inhibition of the mechanism at that time could thereby attenuate subsequent calcium overload.³² Calcium overload is a suspected mechanism of the transition from reversible to irreversible injury. The reduction in neutrophil accumulation in the area at risk with cariporide treatment is consistent with a generalized reduction in the inflammatory response to ischemia-reperfusion¹⁶ rather than with a direct antineutrophil effect. Numerous studies have reported a correlation between attenuated neutrophil accumulation and reduced postischemic injury in both surgical and nonsurgical models of ischemia-reperfusion.¹⁶

Previous studies have demonstrated that NHE-1 is the sole isoform detectable in the cardiac myocyte³⁶ and that the activity of the NHE is responsible for approximately 50% of extrusion of hydrogen ion equivalents in cardiac tissue.³⁷ Cariporide inhibits more than 95% of the NHE-1 activity at 1 $\mu\text{mol/L}$ concentration. Inhibition of the NHE-1 mechanism during myocardial ischemia and reperfusion delays realkalinization to normal intracellular pH^{9,38} and prevents subsequent calcium overload and its deleterious consequences in cardiomyocytes.^{32,38} We did not measure intracellular pH or intracellular calcium in this study, but we speculate that the inhibition of calcium entry and accumulation occurred primarily at the time of aortic crossclamping and at reperfusion.

We found that recovery of postischemic segmental function was not significantly improved by cariporide, despite dramatic reductions in infarct size and myocardial edema. Segmental work at 90 and 120 minutes of reperfusion strongly tended to be greater in the group treated with cariporide blood cardioplegia, whereas there was no difference in segmental shortening. This suggests that there was

Figure 5. Vasorelaxation responses to incremental concentrations of acetylcholine (*left*) and sodium nitroprusside (*right*) in both occluded-reperfused LAD (*squares*) and nonischemic LCx (*circles*) coronary artery rings. **A,** Vehicle (*Veh*) group. **B,** Cariporide (*NHEI*) group. Asterisk designates *P* value versus vehicle group at same concentration.

still systolic bulging in the ischemic-reperfused myocardium, but shortening occurred during late systole. This failure to restore contractile function was also reported for previous studies in which the NHE inhibitor was administered after the onset of coronary occlusion.^{14,15,39} However, models of global ischemia have reported salutary effects. Reversible global ischemia may involve myocardial stunning, as opposed to the infarction observed with more prolonged periods of regional occlusion. However, it is not clear whether long-term recovery would demonstrate benefit, because most studies were conducted in acute (hours of reperfusion) models.

In summary, the present study demonstrated that blood cardioplegia supplemented with the NHE inhibitor cariporide provided superior cardioprotection following normothermic regional myocardial ischemia when used as an adjunct to blood cardioplegia. The protective effect was likely exerted directly on the myocytes since the concentration used was less than concentrations that directly inhibit neutrophil function. However, the attenuation of neutrophil accumulation and endothelial dysfunction most likely represent a reduced inflammatory response to ischemia-reperfusion. The effectiveness of hypothermic blood cardioplegia supplemented with such purported cardioprotective agents is predicated on the ability of the agent of interest to exert

its therapeutic effects during the cardioplegic period or thereafter on the end points measured. In previous clinical trials of NHE inhibitors, such as the GUARDIAN Trial,²⁰ cariporide was administered intravenously to high-risk patients before cardiac surgery and for as long as several days perioperatively. However, the greatest risk for the patient occurs in the immediate postoperative period, the events of which may be influenced by intraoperative events and the adequacy of myocardial protection. Incorporation of a NHE inhibitor only at the time of cardioplegia delivery is a strategy that has not been tested yet in clinical trials. This study provides a scientific foundation supporting the use of NHE inhibitors during the intraoperative period as an adjunct to clinical cardioplegia. However, it is not known whether continued postischemic administration would have resulted in further cardioprotection. In addition, further studies should be pursued to determine whether use of a higher concentration of cariporide to directly inhibit neutrophil superoxide radical generation would increase the degree of myocardial protection.

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