Myocyte and endothelial effects of preconditioning the jeopardized heart by inhibiting Na^+/H^+ exchange

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Objectives: The preconditioning effects of the adjunctive, cardiac-specific sodium-hydrogen ion exchange inhibitor cariporide (cariporide mesilate, HOE 642) were studied in hearts subjected to 30 minutes of normothermic ischemia and reperfusion to assess myocardial and endothelial changes.

Methods: Sixteen Yorkshire-Duroc pigs (27-34 kg) receiving cardiopulmonary bypass underwent either cardiopulmonary bypass alone (control, $n = 4$) or 30 minutes of normothermic ischemia, followed by 30 minutes of blood reperfusion ($n = 12$). Six hearts were treated with 5 mg/kg cariporide administered intravenously 15 minutes before ischemia.

Results: Cardiopulmonary bypass alone caused no changes. Conversely, 30 minutes of global normothermic ischemia caused 33% mortality and, in survivors, depression of left ventricular function to $22\% \pm 6\%$ of baseline preload recruitable stroke work and increased creatine kinase MB by 406% (88 ± 13 U/L), conjugated dienes by 17% (161 ± 0.2 AU/mL), and myeloperoxidase activity by 297% (0.036 ± 0.005 U/g). Myocardial edema developed (3.5% water gain). Coronary sinus endothelin 1 increased by 111% (2.05 ± 0.38 pg/mL), and nitric oxide production decreased by 10%. These adverse effects were limited by pretreatment with cariporide, which allowed complete survival and restored preload recruitable stroke work to $78\% \pm 11\%$. Measurements of creatine kinase MB, conjugated dienes, myeloperoxidase, water, and endothelin 1 returned to baseline values, and nitric oxide production was accentuated 3-fold.

Conclusions: These observations show that adjunctive pretreatment with cariporide delays myocardial and endothelial injury during ischemia and reperfusion, limits oxygen-derived radical injury, restores function, reduces edema, and preserves endothelin and nitric oxide balance at normal values. The myeloperoxidase changes show that less white blood cell adherence supports reduced reperfusion endothelial damage.

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The hemodynamic consequence of ischemic damage followed by normal blood reperfusion is closely related to that of calcium overload. The resultant injury includes cell membrane damage, impaired endothelial function with endothelin release, and limited nitric oxide (NO) elaboration, resulting in leukocyte adherence, oxidant injury, and compromised pump function.

The genesis of reperfusion-related injury is linked to alteration of at least 3 calcium-related fundamental factors that include (1) the Na^+/H^+ exchange mechanism, which is inactive during normal perfusion, but during ischemia extrudes pH protons for sodium in an electron-neutral exchange that becomes the main buffering system of the cell; (2) the adenosine triphosphate (ATP), energy-dependent sodium-potassium exchange that extrudes sodium to prevent cellular overload; and (3) the

energy-independent $\text{Na}^+/\text{Ca}^{++}$ exchanger that normally extrudes calcium to avoid intracellular overload. Prolonged ischemia (>5 minutes) accentuates acidosis and modifies these protective factors by interfering with the ATP-dependent sodium-potassium pump and activating the sodium-hydrogen exchanger. The resultant sodium accumulation allows subsequent calcium overload by means of unbridled and now reversed activity of the sodium-calcium exchanger. Calcium now enters the cell to reduce intracellular sodium accumulation and accentuates damage.¹ Secondary water accumulation follows sodium overload, resulting in edema that impairs compliance and restricts flow,² ultimately contributing to the death of myocytes that otherwise might have survived reperfusion.

Prior studies have focused on calcium-channel blocking agents that limit calcium entry at the membrane level with drugs that might have adverse long-term effects (ie, nifedipine). Novel management concepts have emerged after recognition that inhibition of the Na^+/H^+ ion exchange mechanism can alter the pathophysiology of ischemia-reperfusion injury. Pretreatment interference of Na^+/H^+ ion exchange activity will maintain intracellular acidosis and thus limit sodium accumulation during the impaired activity of the ATP-dependent sodium-potassium pump during the ischemia-reperfusion interval. These have been isolated studies^{1,3,4-7} rather than studies in the intact animal that reflect the conditions relating to extracorporeal circulation by using a model of a severely damaged heart for comparison. The resultant reduced sodium accumulation restricts the progressive detrimental activity of the sodium-calcium exchanger. Cellular calcium overload is prevented until more normal ion transfer mechanisms become restored during the recovery phase of reperfusion.

The normal Na^+/H^+ exchange function is most useful during transient ischemia because controlling intracellular pH will sustain contraction by maintaining electroneutral exchange with hydrogen. Unfortunately, continuing activation during prolonged ischemia might cause calcium-related hypercontracture if sodium can not be extruded during reperfusion. Reversal of this energy-dependent impairment does not occur immediately after reperfusion⁸ because the operative adverse mechanism is ongoing depression of the Na^+/K^+ pump from impaired ATP genesis.⁹ This observation defines a paradox because Na^+/H^+ exchange inhibition will maintain acidosis and is a marked contrast to conventional efforts to rapidly reverse acidosis during the immediate interval of reperfusion.

Cariporide (HOE 642) is a specific inhibitor of the Na^+/H^+ exchanger that delays myocardial damage^{1,3-5} and reduces arrhythmias^{4,6,7} experimentally and lowers mortality and incidence of infarction in high-risk patients undergoing coronary artery bypass grafting.¹⁰ Intraoperative myocardial protection methods (ie, cardioplegia) were ex-

tensively varied in this clinical report, so that direct quantification of pretreatment capacity could not be established. These early positive clinical benefits of Na^+/H^+ exchange inhibition set the stage for our study, which aims at evaluating the pretreatment ability of the drug alone. Testing was done in vulnerable hearts independent of adding the variable of cardioplegic protection.

We selected an injury model that causes contractile and endothelial dysfunction to simulate high-risk patients.¹¹ This dual injury involves endothelin 1 (ET-1) release and impaired NO production, with resultant vascular leukocyte adherence and secondary cytotoxic oxygen radical production.^{12,13} These vascular effects can compound contractile dysfunction and should be offset by pretreatment. The guidelines for Na^+/H^+ inhibition success are improvement of mechanical function, cellular and oxidant injury, leukocyte adherence, and the vascular balance of endothelin and NO levels.

Material and Methods

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the Institute of Laboratory Animal Resources and the "Guide for the Care and Use of Laboratory" prepared by the National Institutes of Health (publication No. 86-23, revised 1985).

Sixteen Yorkshire-Duroc pigs (27-34.5 Kg) were premedicated (15 mg/kg ketamine and 0.5 mg/kg diazepam administered intramuscularly), and anesthesia was achieved with 30 0.5 mg/kg pentobarbital administered intravenously and subsequent bolus injections of sodium pentobarbital. Support with a volume-controlled ventilator (Servo 900C, Siemens-Elcoma) was started after tracheostomy and endotracheal intubation. The femoral artery and vein were cannulated, and arterial blood gases were measured to maintain oxygen tension, carbon dioxide tension, and pH values within the normal range. A balloon-tipped pulmonary artery catheter (Model 132F5, Baxter Healthcare Corp) measured cardiac output (thermodilution technique) and pulmonary artery pressure.

After a median sternotomy and pericardial incision, a solid-state pressure transducer-tipped catheter (Model MPC-500, Millar Instruments, Inc) was apically placed to monitor left ventricular (LV) pressure. Left ventricle major and minor axes were measured with endocardially placed 2-mm ultrasonic microtransducer crystals (Sonometrics). LV volume was assessed by using an ellipsoid-based formula. Pressure-volume loops were recorded digitally with acquisition hardware and software (Sonometrics).

After systemic heparinization (300 U/kg), a 12F aortic cannula was inserted in the ascending aorta, and a dual-lumen 29F venous cannula was inserted in the right atrium through the right appendage. Extracorporeal circulation with a membrane oxygenator (Affinity NT 541, Medtronic, Inc) and extracorporeal pump (Sarns) included a circuit primed with 1000 mL of Plasma-Lyte solutions (Baxter Healthcare Corp), 700 mL of stored porcine packed blood, and calcium chloride for normocalcemia (1.0-1.2 mmol/L).

Cardiopulmonary bypass (CPB) was started at an oxygen tension of 300 mm Hg and an aortic pressure of 50 to 70 mm Hg, adjusting flow to maintain approximately 70% mixed venous oxygen saturation. Potassium, calcium, and pH were kept at normal

levels. A dual-lumen aortic cannula in the aortic root measured delivery of blood and aortic root pressure. Transatrial coronary sinus cannulation allowed blood sampling, and the left ventricle was vented. Rectal temperature was maintained at 35°C to 37°C during extracorporeal circulation. All cases were performed and analyzed by the same surgeon.

Experimental Protocol

Pigs were nonrandomly divided into 3 groups.

Nonischemic control hearts. The nonischemic control group was composed of 4 piglets that underwent 60 minutes of normothermic CPB to distinguish the effects of extracorporeal circulation alone without ischemia.

Ischemic hearts. In 12 hearts ischemic damage was produced by inducing 30 minutes of normothermic aortic clamping. Extracorporeal circulation was maintained for 30 minutes after unclamping and before measuring biochemistry and function.

In ischemic hearts without preconditioning (group 1), in 6 studies reperfusion was performed with normal blood from the extracorporeal circuit. In ischemic hearts with preconditioning (group 2), in 6 pigs 5 mg/kg cariporide (HOE 642) was added to the extracorporeal circuit 15 minutes before inducing ischemia. The cariporide half-life is 30 minutes, and therefore blockage of the sodium-hydrogen exchanger was obtained with this dosage regimen.

Mechanical Measurements

Global LV function before and 30 minutes after CPB was assessed by means of Starling curves¹⁴ and pressure-volume analysis.¹⁵ Cardiac output was determined by using duplicate central venous injections of 3 mL of 4°C saline solution, and the LV stroke work index (LVSWI) was calculated by using the following formula:

$$\text{LVSWI} = (\text{MAP} - \text{LAP}) \times \text{CO} \times 0.0136 \times \text{HR}^{-1} \times \text{Weight} - 1$$

where MAP indicates mean arterial pressure, LAP indicates left atricular pressure, and HR indicates heart rate.

Modified Starling curves increased during preload conditions by continuously infusing blood intravenously at $4 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ during pacing at 150 beats/min during cardiac output. Afterload was not controlled, but these transfusions simulated clinical volume loading, with stroke work index analysis achieved by recording cardiac output and blood pressure.

Pressure-volume curves evaluated cardiac performance with a preload-, afterload-, and heart rate-independent method. Measurements of LV pressure and volume were recorded during transient inferior vena cava occlusions to obtain a series of evenly declining pressure-volume loops. Global stroke work and end-diastolic volume were calculated with a video graphics program (SPECTRUM, Triton Technology). Preload recruitable stroke work (PRSW) for each series was identified as the relation between stroke work and end-diastolic volume and quantified by using a slope and x-intercept. The slope ($\text{erg} \times \text{cm}^3 \times 10^3 \text{ mW/cm}^3$ of myocardium) measures myocardial performance independent of loading, geometry, and heart rate.^{16,17} Postbypass LV performance was expressed as a percentage of recovery from prebypass values.

Biochemical Analysis

Coronary sinus blood samples were taken 5 minutes after initiating CPB (baseline) and 30 minutes after initiation of reperfusion. Both

samples were obtained in CPB to maintain the same degree of hemodilution. During these measurements, coronary blood flow was maintained at 100 mL/min (by using controlled aortic perfusion with normal blood during transient aortic clamping) as plasma samples were analyzed.

Conjugated dienes. As a marker of oxidant-mediated lipid peroxidation, conjugated diene (CD) levels were determined spectrophotometrically after chloroform-methanol 2:1 (vol/vol) extraction, as previously described,¹⁸ and expressed as absorbance at a wavelength of 240 nm per 0.5 mL of plasma.

Creatine kinase MB. Myocardial damage was determined by measuring creatine kinase (CK) fraction MB (in units per liter) with a UV-spectrophotometric method (Sigma Chemical Co), as recommended by the German Society for Clinical Chemistry.

Nitric oxide. NO (in micromoles per liter) was determined as its spontaneous oxidation products, nitrite and nitrate, which were converted to NO and quantitated by using a Chemiluminescence assay with a nitrogen oxide analyzer (Model 2108, DASIBI Environmental Corp). We measured venous levels and compared these over time. Our pilot studies showed no significant arterial-venous gradient, probably because of negligible release.

Endothelin 1. ET-1 levels (in picograms per milliliter) were determined after sample purification (Ethyl C2 Amprep minicolumns, Amersham Pharmacia Biotech) by using an Enzyme Immunoassay (ACE EIA kit, Cayman Chemical Co) on the basis of a double-antibody sandwich technique.

Myocardial Biopsy

Final measures of water content and myeloperoxidase activity were made on hearts harvested after termination by means of bolus injection of 5 mg of pentobarbital, followed a minute later by 15 mL of cold hyperkalemic blood (KCL, 30 mEq/L).

Myeloperoxidase activity. Transmural samples of the anterior free wall of LV myocardium (approximately 0.5 g) were immediately frozen in liquid nitrogen, subsequently analyzed for neutrophil-specific myeloperoxidase activity, and expressed in units per gram of tissue.¹⁹

Water content. Myocardial tissue was weighed before and after incineration to determine the tissue percentage of water content.

Statistical Analysis

Statistical analysis of data within and between groups was performed by using multiple analysis of variance, followed by application of the Student *t* test with the Tukey-Kramer correction for multiplicity. All data are expressed as means $\pm$ SD.

Results

LV Performance

Global volume loading of control hearts (without ischemic damage) did not change the prebypass LVSWI after transfusion to 15 mm Hg left atrial pressure, as seen in Figure 1. In contrast, 2 ischemic hearts (without preconditioning) could not sustain sufficient output to enable weaning from extracorporeal circulation, and the other 4 pigs recovered only $25\% \pm 5\%$ of baseline LVSWI value. Pretreatment

Figure 1. Postischemic LV performance assessed by means of Starling curves. *SWI*, Stroke work index; *LAP*, left atrial pressure.

with cariporide allowed all piglets to be weaned from CPB, with LVSWI recovery of $67\% \pm 11\%$.

There were no significant differences in baseline PRSW values between groups (mean of all groups, $78 \pm 23 \text{ erg} \cdot \text{cm}^{-3} \cdot 10^3$). In control studies without ischemia, PRSW returned to $105\% \pm 4\%$ ($99 \pm 37 \text{ erg} \cdot \text{cm}^{-3} \cdot 10^3$) of baseline values (Figure 2). In 4 of 6 survivors of ischemia without pretreatment, PRSW returned to $22\% \pm 6\%$ ($19 \pm 8 \text{ erg} \cdot \text{cm}^{-3} \cdot 10^3$) of baseline values. Conversely, 6 of 6 cariporide pretreatment survivors recovered to $78\% \pm 11\%$ ($57 \pm 15 \text{ erg} \cdot \text{cm}^{-3} \cdot 10^3$) of baseline levels ($P < .05$).

Myocardial Damage

In nonischemic hearts CK-MB levels remained within the normal range ($<25 \text{ U/L}$, Figure 3). Normothermic ischemia without pretreatment increased the coronary sinus plasma levels to $88 \pm 13 \text{ U/L}$, whereas pretreated CK-MB increased to $42 \pm 4 \text{ U/L}$ ($P < .05$).

CD levels remained within the normal range at $1.22 \pm 0.02 \text{ AU/0.5 mL}$ in control hearts. Ischemic injury (Figure 4) increased CDS to $1.61 \pm 0.15 \text{ AU/0.5 mL}$ ($P < .05$ vs control). Pretreatment with cariporide decreased CDS to $1.36 \pm 0.10 \text{ AU/0.5 mL}$, which was significantly less than seen during ischemia without pretreatment.

Myeloperoxidase activity, marking white blood cell injury (Figure 5), increased approximately 4-fold without cariporide pretreatment. In contrast, pretreatment caused a marginal augmentation versus control values ($P < .05$), with values significantly less than those after ischemia without cariporide ($P < .05$).

Extracorporeal circulation (Figure 6) caused an insignificant increase in water content, whereas normothermic ischemia caused a marked increase to $82.3\% \pm 0.5\%$ water content ($P < .05$ vs bypass alone) that was prevented by

cariporide pretreatment ($80.5\% \pm 0.5\%$, $P < .05$ vs ischemic group).

Endothelial Response

Extracorporeal circulation without ischemia caused a slight increase in endothelin versus baseline values, as shown in Figure 7. In contrast, ischemia doubled endothelin to $2.05 \pm 0.38 \text{ pg/mL}$, whereas cariporide pretreatment endothelin values were similar to levels achieved with extracorporeal circulation alone (1.22 ± 0.15 vs $1.25 \pm 0.07 \text{ pg/mL}$).

NO levels after nonischemic CPB were similar to baseline values (Figure 8) and were unchanged by ischemia. Significant augmentation to $270\% \pm 173\%$ followed cariporide pretreatment and produced higher levels than those after ischemia ($P < .05$), with moderate intergroup variability.

Discussion

The endothelial and myocyte damage produced by 30 minutes of normothermic arrest includes leakage of CK-MB, oxygen radical injury, water accumulation, endothelin release, and functional damage, followed by normal blood supply replenishment. These adverse effects were substantially limited by means of pretreatment with cariporide, an Na^+/H^+ exchange inhibitor.

We found that cariporide pretreatment (15 minutes before aortic clamping) diminished the mechanical effect of profound ischemia-reperfusion injury because Starling curves and PRSW (an index more independent of load, heart rate, or geometry)^{16,17} showed marked recovery compared with 33% mortality and profound depression in survivals without pretreatment. The early maintenance of an acidotic, rather than immediate, buffering to a normal pH level was helpful and did not impair membrane function because less

Figure 2. Recovery of PRSW expressed as a percentage of preischemic control values. Data are expressed as means $\pm$ SD. * $P < .05$ versus the control group and group 2; ** $P < .05$ versus the control group and group 1.

Figure 3. Levels of CK-MB in units per liter from coronary sinus plasma at baseline and after 30 minutes of reperfusion. Data are expressed as means $\pm$ SD. * $P < .05$ versus group 2 and the control group.

cytosolic enzymatic leakage of CK-MB occurred in the pretreated group ($P < .05$) and phospholipid injury was diminished.

Our model of ischemia-reperfusion damage might mirror the vulnerability of high-risk patients who must undergo CPB. Although this independent evaluation of pretreatment interventions in injured hearts was introduced before adding conventional myocardial protection studies (ie, hypothermia and cardioplegia), subsequent studies must be done with the aforementioned cardioplegic protection methods.

The Na^+/H^+ exchanger is the natural system to extrude protons out of the cell. It is activated by intracellular acidosis and by various autocrine and paracrine factors, such as ET-1, angiotensin II, α_1 -adrenergic agonists, and toxic agents, such as hydrogen peroxide and lysophosphatidylcholine.²⁰ Its activation during short periods of ischemia (<5 minute) allows continuing contraction by limiting an-

aerobic-induced lactacidosis. After prolonged ischemia, contractility stops, and reperfusion damage becomes an important pathophysiologic factor from intracellular Ca^{++} overload.^{1-4,9} We ascribe the beneficial effects of Na^+/H^+ exchange blocker to limiting excessive Ca^{++} influx during ischemia and reperfusion. This accumulation occurs after Na^+ overload, resulting from impaired capacity to exclude sodium through ischemic damage of the ATP-dependant sodium-potassium pump and the Na^+ influx by the sodium-hydrogen exchanger. Consequently, the energy-independent $\text{Na}^+/\text{Ca}^{++}$ pump becomes reversed because of a loss of cohesion with the ATP-dependent means of extruding sodium, and calcium accumulates and can become massive. These adverse events of ongoing activity of the Na^+/H^+ exchanger, responsible for Na^+ influx, are implicated as the mechanism of reperfusion injury in multiple experimental studies.^{1,3,4} Conversely, inhibition of the exchanger by ca-

Figure 4. Levels of CDS in coronary plasma expressed as absorbance units (A) per 0.5 mL at baseline and after 30 minutes of reperfusion. Data are expressed as means $\pm$ SD. * $P < .05$ versus group 2 and the control group.

Figure 5. LV anterior free wall myocardial myeloperoxidase activity taken at the end of the reperfusion period. Data are expressed as means $\pm$ SD. * $P < .05$ versus group 2 and the control group.

riporide during ischemia will attenuate intracellular accumulation of Na^+ and Ca^{2+} in cardiomyocytes and diminish rigor contracture during ischemia and reperfusion.⁴

The beneficial effects of cariporide during regional ischemia include reduction of infarct size,^{4,21} improved post-myocardial infarction LV function,²² reduced apoptosis,²³ reduced ATP preservation,²⁴ and reduced auricular and ventricular arrhythmias during reperfusion by reducing the refractory period.^{6,7} In the surgical setting cariporide increases tolerance to global ischemia and reperfusion,²¹ can be given before ischemia, and might become more efficient than

Figure 6. LV anterior free wall myocardial water content expressed as a percentage of weight taken at the end of the reperfusion period. Data are expressed as means $\pm$ SD. * $P < .05$ versus group 2 and the control group.

ischemic preconditioning.²⁵ Our surgical model differs from regional ischemia by (1) controlling the interval of damage (vs unstable angina or percutaneous transluminal coronary angioplasty after myocardial infarction) and (2) recognizing that subsequent added protective mechanisms (ie, hypothermia and cardioplegia) can be used during surgical repair.

The contractile recovery after Na^+/H^+ exchange blockade (Figure 9) was associated with the secondary effects of limiting reperfusion edema that might reflect less Na^+ gain after cariporide pretreatment. These mechanical effects of the Na^+/H^+ exchange blockade are well known, but novel endothelial-related benefits (quantified by our measuring NO and endothelin) might parallel reduced myocardial stunning and edema and could expand how Na^+/H^+ exchange blockade alters ischemia-reperfusion damage.

Figure 7. Levels of ET-1 from coronary sinus plasma at baseline and after 30 minutes of reperfusion. Data are expressed as means $\pm$ SD. * $P < .05$ versus group 2 and the control group.

Figure 8. Change in NO production as a percentage from baseline levels. Data are expressed as means $\pm$ SD. * $P < .05$ versus group 1.

Figure 9. Pressure-volume loops (*above*) and stroke work end-diastolic area (PRSWI) (*below*) in hearts under baseline conditions (A), after ischemia (B), and after ischemia and pretreatment (C).

The vascular endothelial-related injury might reflect an improved balance between retained NO production capacity to support vasodilatation (shown by the increase in NO levels) and avoidance of increased endothelin production, which enhances vasoconstriction. We cannot define precisely whether this was a predominant endothelial effect because neither endothelium nor myocardium underwent biopsy, and thus the evidence only suggests this relationship. The Na^+/H^+ blockade aided both factors in this endothelin-NO relationship, but our observational studies did

not search for the responsible mechanisms. We believe that the endothelial NO-endothelin relationship (a modular balance between vasodilatation and vasoconstriction) might reflect a novel vascular harmony. A similar but recognized relationship exists in myocytes, where the supply-demand balance modulates normal muscle function under physiologic conditions.

The endothelial protection responsibilities of NO include maintaining vascular dilatation, avoiding adhesion and aggregation of platelets and neutrophils, promoting dissolu-

tion of platelet aggregates, and secondarily preserving myocardial mechanical and electrical activity.^{12,13,26} NO levels were increased in pretreated hearts (Figure 8). The adverse effects of postischemic endothelial cell dysfunction are also associated with decreased endothelium-dependent vasodilation and ET-1 elevation.²⁷ ET-1 release produces reperfusion endothelial-dependent vascular and nonvascular smooth muscle contraction that exacerbates ischemic contraction and direct myocardial death.²⁷⁻²⁹

Neutrophil activation worsens ischemia-reperfusion injury because endothelial cells develop several binding neutrophil sites for attachment and penetration during reperfusion,³⁰ leading to obstruction of reflow and oxygen radical damage. Myeloperoxidase activity reflects leukocyte accumulation in the tissue assayed and was lower in treated versus untreated hearts. This was associated with less lipid peroxidation, which was assessed by measuring CDS. Such pretreatment benefits differ from direct reperfusion tactics that minimize deleterious effects of neutrophil activation by using leukocyte depletion filters,³¹ by means of pharmacologic alteration of leukocyte function,³² or by adding exogenous oxygen radical scavengers for evolved lipid peroxidation products.³³

Independent endothelin augmentation can unbalance the NO-endothelin relationship and accentuate ischemia-reperfusion injury, even with normal NO production, and become accentuated when NO production decreased.¹³ Our prior studies suggest that NO synthesis is not impaired because exogenous precursors for NO synthesis, such as L-arginine, amplify coronary sinus NO levels.¹¹

Cariporide preserves the balance between endothelin and NO compared with that of untreated hearts, as shown in Figures 7 and 8. This unanticipated limitation of endothelin release might relate to the inhibitory effects of cariporide on calcium concentration¹ and emphasizes that Na^+/H^+ exchange blockade might positively contribute to potential recovery of both endothelium and muscle in the ischemia-reperfusion injury process. The favorable vascular results of pretreatment suggest that an anti-inflammatory action³⁴ might supplement endothelial and myocardial effects.

We conclude that cariporide (1) delays direct myocardial and endothelial injury in the ischemia-reperfusion model when administered before the onset of ischemia and (2) also might indirectly diminish myocardial and endothelial injury by maintaining a more normal endothelin-NO balance, limiting oxygen radical damage and endothelium leukocyte binding and thereby protecting the endothelium, as well as the myocytes. These pretreatment studies in the jeopardized myocardium set the stage for future evaluation for pretreatment of damage in preoperative high-risk ventricles that must undergo more subsequent prolonged surgical aortic clamping to repair the underlying defect.

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