






ORIGINAL RESEARCH

ATP—Sensitive Potassium Channel Opener Diazoxide Reduces Myocardial Stunning in a Porcine Regional With Subsequent Global Ischemia Model

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BACKGROUND: ATP-sensitive potassium channels are inhibited by ATP and open during metabolic stress, providing endogenous myocardial protection. Pharmacologic opening of ATP potassium channels with diazoxide preserves myocardial function following prolonged global ischemia, making it an ideal candidate for use during cardiac surgery. We hypothesized that diazoxide would reduce myocardial stunning after regional ischemia with subsequent prolonged global ischemia, similar to the clinical situation of myocardial ischemia at the time of revascularization.

METHODS AND RESULTS: Swine underwent left anterior descending occlusion (30minutes), followed by 120minutes global ischemia protected with hyperkalemic cardioplegia±diazoxide (N=6 each), every 20minutes cardioplegia, then 60minutes reperfusion. Cardiac output, time to wean from cardiopulmonary bypass, left ventricular (LV) function, caspase-3, and infarct size were compared. Six animals in the diazoxide group separated from bypass by 30minutes, whereas only 4 animals in the cardioplegia group separated. Diazoxide was associated with shorter but not significant time to wean from bypass (17.5 versus 27.0minutes; $P=0.13$), higher, but not significant, cardiac output during reperfusion (2.9 versus 1.5L/min at 30minutes; $P=0.05$), and significantly higher left ventricular ejection fraction at 30minutes (42.5 versus 15.8%; $P<0.01$). Linear mixed regression modeling demonstrated greater left ventricular developed pressure ($P<0.01$) and maximum change in ventricular pressure during isovolumetric contraction ($P<0.01$) in the diazoxide group at 30minutes of reperfusion.

CONCLUSIONS: Diazoxide reduces myocardial stunning and facilitates separation from cardiopulmonary bypass in a model that mimics the clinical setting of ongoing myocardial ischemia before revascularization. Diazoxide has the potential to reduce myocardial stunning in the clinical setting.

Key Words: animals ■ cardiopulmonary bypass ■ diazoxide ■ heart arrest, induced ■ myocardial stunning ■ swine ■ ventricular function, left

The underlying mechanism for the cardioprotective action of ATP potassium (K_{ATP}) channel openers remains elusive. It has been proposed that diazoxide and other K_{ATP} channel openers provide benefit via a mechanism of mitochondrial matrix expansion.^{1–3} Opening of a mitochondrial K_{ATP} would

result in K^+ influx from the cytosol into the mitochondria⁴ and mitochondrial swelling, suggesting a potential basis for mitochondrial K_{ATP} -dependent changes in mitochondrial and cellular volume secondary to stress. We have shown that diazoxide does not provide cardioprotection via sarcolemmal K_{ATP} channels,⁵

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Nonstandard Abbreviations and Acronyms

CPB	cardiopulmonary bypass
dp/dt max	maximum change in ventricular pressure during isovolumetric contraction
dp/dt min	maximum change in ventricular pressure during isovolumetric relaxation
K_{ATP}	ATP potassium
LVDP	left ventricular developed pressure

although it requires the K_{ATP} channel subunit sulfonyleurea receptor¹⁵ and requires the inhibition of succinate dehydrogenase.⁶

We have demonstrated that K_{ATP} channel openers prevent the detrimental consequences of various stresses in isolated animal and human myocytes.^{7–10} We have uniquely linked myocyte volume with contractility (demonstrating an inverse relationship following stress), and thus identified a cellular etiology (loss of volume homeostasis) as one mechanism of myocardial stunning.^{7,11} Similarly, we developed isolated mouse heart and intact porcine models of prolonged global ischemia mimicking the clinical situation of global ischemia during cardiac surgery and demonstrated that diazoxide provides additional cardioprotection when added to traditional hyperkalemic cardioplegia versus cardioplegia alone.^{12,13} Using these models, we have characterized this myocardial protection and demonstrated the potential for K_{ATP} channel opener use in humans undergoing cardiac surgery.

The majority of patients who undergo cardiac surgery with cardiopulmonary bypass (CPB) with global ischemia (during cross-clamp application) exhibit myocardial stunning despite efficient electromechanical arrest.¹⁴ The myocardium is subject to the added stresses of global ischemia with exposure to hypothermic, hyperkalemic cardioplegia (known to independently result in reduced myocyte contractility and function).¹⁵ Myocardial stunning following these insults requires inotropic and mechanical support following cardiac surgery. Pharmacologic K_{ATP} channel openers such as diazoxide may have the potential to prevent stunning, improve patient outcomes, and reduce health care costs.

The hypothesis for the current study was that the addition of diazoxide to hypothermic hyperkalemic cardioplegia would reduce myocardial stunning after 30 minutes of left anterior descending artery (LAD) occlusion (regional ischemia), followed by 2 hours of global ischemia compared with cardioplegia alone. The large animal model used uniquely simulates the situation of a patient presenting to the operating room for coronary revascularization with ongoing myocardial ischemia.

METHODS

Operative Protocol

The Johns Hopkins Animal Care and Use Committee approved of this study, and animal care was conducted according to their guidelines. Eighteen American Yorkshire swine (38–43 kg) were included: 9 male and 9 female. The operative protocol is represented in [Figure 1](#). Animals were sedated using intramuscular telazol (4.4 mg/kg), ketamine (2.2 mg/kg), and xylazine (2.2 mg/kg) and intubated as previously described.¹³ After intubation, anesthesia was maintained with isoflurane. Systemic temperature was monitored with a rectal probe and maintained between 35 and 37 °C by using a warm water blanket.

The left common carotid artery and internal jugular vein were cannulated for blood pressure monitoring and administration of fluids and medications. After sternotomy, 300 U/kg of heparin was given. Pacing wires were placed in the right atrium for pacing at 95 bpm. After confirming adequate heparinization (>480 activated clotting time [ACT]), the aorta was cannulated. Antegrade and retrograde cardioplegia catheters were inserted in the aorta and in the coronary sinus, respectively, for cardioplegia delivery. The LAD was occluded just distal to the second diagonal branch using a vessel loop and a tourniquet. Adequate occlusion was confirmed by visualization of a change of color in the apex ([Figure 2](#)). After 20 minutes of regional ischemia, a dual-stage venous cannula was placed in the right atrial appendage. After 30 minutes of regional ischemia, CPB was initiated and the aorta was cross clamped for 2 hours. All researchers performing each experiment remained blinded to group allocation. Hypothermic hyperkalemic blood cardioplegia (modified St. Thomas solution, 106 mEq K/L) was delivered in an antegrade (500 mL) and then retrograde fashion (500 mL). Animals were randomly assigned to receive an induction dose of cardioplegia with diazoxide (500 μmol/L) or cardioplegia alone (control group). The dose used was determined by a dose-response curve in prior work and is the same that has been tested in previous mouse and swine experiments.^{12,13,16} Low-dose (62 mEq K/L) retrograde hypothermic hyperkalemic cardioplegia (300 mL) was administered every 20 minutes throughout 2 hours of global ischemia in both groups. After 2 hours of ischemia, lidocaine (100 mg) and magnesium sulfate (2.5 mg) were administered, the aortic cross clamp was removed after appropriate deairing maneuvers, and the tourniquet on the LAD was removed. Animals were then weaned off CPB using norepinephrine (0.01–0.40 μg/kg per min), phenylephrine boluses (0.2 mg), or defibrillation as needed. After data collection and 1 hour of reperfusion, potassium was administered intravenously under anesthesia for euthanasia. Cardiectomy was performed, and the left

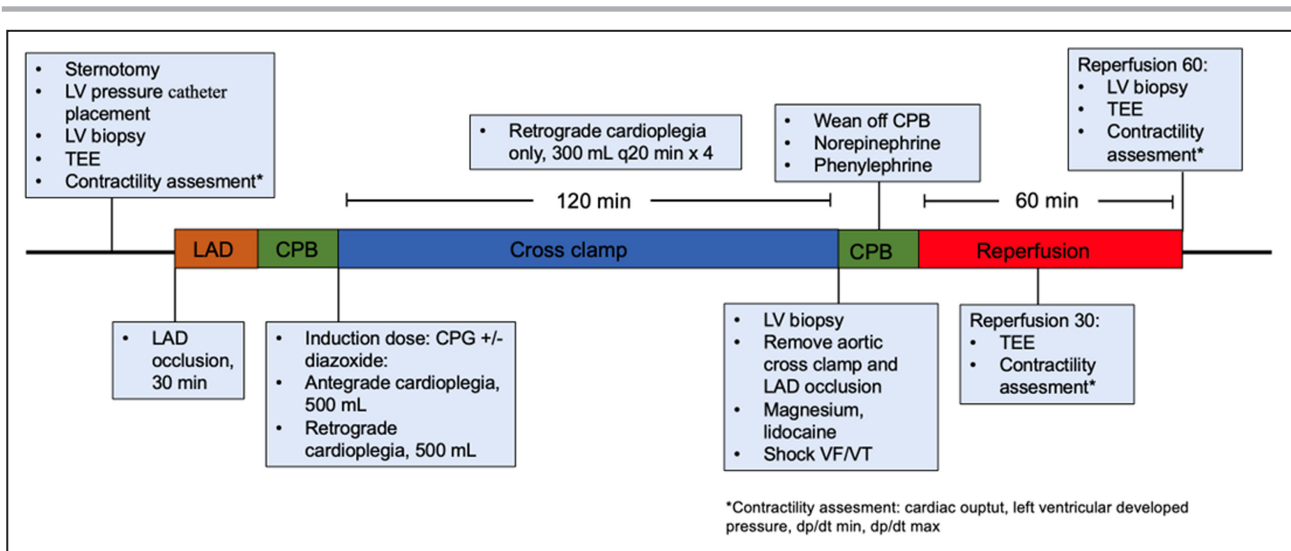


Figure 1. Operative protocol and time points of data acquisition in a porcine model of 30minutes of regional ischemia followed by 2hours of global ischemia.

Animals were randomly assigned to a control group of hyperkalemic cardioplegia alone (N=6) or cardioplegia with diazoxide (N=6). After baseline data acquisition, the LAD was occluded for 30minutes, cardiopulmonary bypass was then instituted, the aortic cross clamp was placed, and cardioplegia \pm diazoxide was administered. During 2 hours of aortic cross-clamp time, low-dose hyperkalemic cardioplegia alone was administered every 20minutes in the retrograde catheter. After 2 hours of global ischemia and removal of the aortic cross clamp and LAD occlusion, efforts to wean from cardiopulmonary bypass were initiated and contractility was assessed throughout 1 hour of reperfusion. CPB indicates cardiopulmonary bypass; CPG, cardioplegia; dp/dt min, maximum change in ventricular pressure during isovolumetric relaxation; dp/dt max, maximum change in ventricular pressure during isovolumetric contraction; LAD, left anterior descending artery; LV, left ventricle; TEE, transesophageal echocardiogram; VF, ventricular fibrillation; and VT, ventricular tachycardia.

ventricle was sectioned and stained with tetrazolium chloride to assess myocardial viability.

Data Acquisition

Arterial blood gas and electrolytes were monitored every 15 minutes. Coronary sinus samples were taken at baseline and after 15, 30 and 60minutes of reperfusion. Myocardial oxygen consumption and cardiac output were calculated using the Fick principle.^{17,18}

Intraventricular pressure was measured using a catheter (Millar Inc., Houston, TX), recorded using LabChart Pro (ADInstruments, Sydney, Australia) and were processed with a blood pressure module (MLS370/8, ADInstruments). Left ventricular developed pressure (LVDP), maximum change in ventricular pressure during isovolumetric relaxation (dp/dt min), and maximum change in ventricular pressure during isovolumetric contraction (dp/dt max) were calculated.

Transesophageal echocardiogram was performed by a blinded cardiac anesthesiologist using a transesophageal sonographic system (Vivid E9 XD Clear and 6VT-D 4D probe, General Electric, Boston, MA). Measurements were recorded at 3 time points: baseline (before LAD occlusion), after 30minutes of reperfusion, and after 60minutes of reperfusion. Left ventricular ejection fraction (LVEF), right and left heart dimensions and mitral velocities were recorded. A

blinded cardiologist interpreted the echocardiographic readings.

Serum samples were collected throughout the experiment, and levels of myoglobin and troponin I were quantified at baseline and after 15, 30, and 60minutes of reperfusion. A small biopsy of the left ventricle was taken from the apex at baseline. Subsequent biopsies were taken after 2 hours of global ischemia and after 60minutes of reperfusion from a region affected by the LAD occlusion (apex) and from a nonaffected region for apoptosis (caspase-3 measurement). Biopsies were fixed in formalin and immunostained.

Upon completion of each experiment, hearts were excised and irrigated with Krebs-Hensleit buffer. The left ventricle was excised, weighed, and sectioned into 5 slices from apex to base. The slices were immersed in a tetrazolium chloride buffer at 37 °C for 15 minutes for myocardial viability assessment as previously reported.^{13,19}

Statistical Analysis

Data from experimental groups were compared using unpaired 2-sided Student's *t* test or Mann-Whitney rank-sum test, as appropriate. A mixed linear regression model was conducted on measurements derived from the intraventricular pressure catheter (LVDP, dp/dt min, and dp/dt max). Because of the large variation in pressure

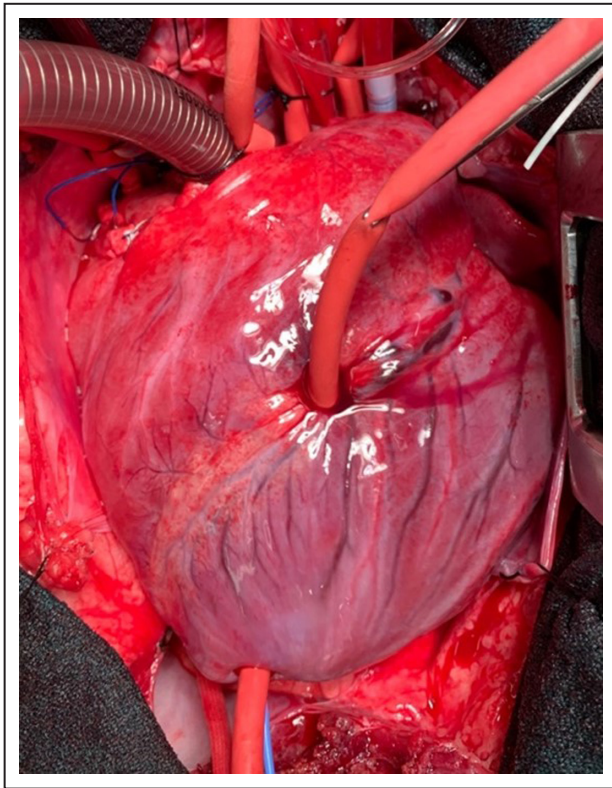


Figure 2. Representative experiment demonstrating the location of the left anterior descending coronary artery occlusion in a porcine model of 30 minutes of regional ischemia followed by 2 hours of global ischemia.

The left anterior descending artery was occluded using a vessel loop and a tourniquet, distal to the second diagonal branch. Adequate occlusion was confirmed by visualization of a change of color in the apex, as demonstrated in this figure. The vessel loop was removed at the time of cross-clamp removal.

measurements within groups, a mixed linear regression model was used to account for these differences. Level of significance was set to $P < 0.05$ for all statistical tests. Analyses were performed using STATA software version 17/SE (StataCorp LLC, College Station, TX).

RESULTS

Ability to Wean From CPB Following 30 Minutes of Regional Ischemia and 2 Hours of Global Ischemia

Six pigs were excluded for the following reasons: presence of severe pericarditis ($n=2$), technical difficulties ($n=3$), and incomplete data capture ($n=1$). Six control swine and 6 diazoxide group swine were included in the study, with an equal distribution of male to female, and a mean weight of 41.8 kg.

Mean systemic temperature during bypass (control, 32.3 °C; diazoxide, 32.4 °C; $P=0.58$) and upon reperfusion (control, 36.9 °C; diazoxide, 36.5 °C; $P=0.42$) was similar between groups.

Animals in the diazoxide group required fewer defibrillations after cross clamp removal, although this was not statistically different between groups (mean number of defibrillations: diazoxide, 5.3; control, 7.5; $P=0.60$; mean number of joules: diazoxide, 122.5; control, 195.8; $P=0.49$). Animals in the diazoxide group received less phenylephrine (2.76 versus 4.27 mg; $P=0.41$) and more norepinephrine (635.8 versus 329.6 μ g; $P=0.11$) than the control group, but the differences were not statistically significant.

After 15 minutes of reperfusion, 3 animals in the diazoxide group and 1 animal in the control group were able to wean from CPB. After 30 minutes of reperfusion, all animals in the diazoxide group ($n=6$) and 4 in the control group weaned from CPB. After 60 minutes, all animals in both groups were off CPB ($n=12$) (Figure 3). The mean time to wean from CPB in the diazoxide group was less than that in the control group, but not statistically significant (mean, 17.5 minutes; SD, 6.89; SEM, 2.81 versus 27 minutes; SD, 13.92; SEM, 5.08, respectively; $P=0.13$).

Myocardial Function Following 30 Minutes of Regional Ischemia and 2 Hours of Global Ischemia

Cardiac Output

Mean cardiac output was higher with diazoxide but not significantly different between groups at baseline or after 15, 30, and 60 minutes of reperfusion (diazoxide group, 1.77, 2.85, 3.19 L/min versus the control group,

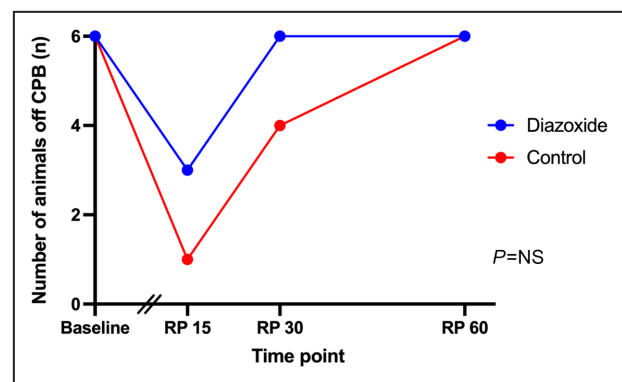


Figure 3. Number of animals that successfully weaned from cardiopulmonary bypass over time.

In a porcine model of 30 minutes of regional ischemia, followed by 2 hours of global ischemia, animals were randomly assigned to a control group of hyperkalemic cardioplegia alone (red line, $N=6$) or cardioplegia with diazoxide (blue line, $N=6$). After 15 minutes of reperfusion, only 1 cardioplegia animal and 3 diazoxide animals had successfully weaned from cardiopulmonary bypass. By 60 minutes of reperfusion, all animals successfully weaned from cardiopulmonary bypass. There were no statistically significant differences in mean time to wean from cardiopulmonary bypass between groups using Student's *t* test. CPB indicates cardiopulmonary bypass; and RP, reperfusion.

0.39, 1.54, 2.46 L/min). There were also no significant differences in percentage change from baseline at any time point. (Table S1, Figure 4).

Myocardial Oxygen Consumption

Myocardial oxygen consumption was not different between groups at baseline, after 15 or 30 minutes of reperfusion. After 60 minutes of reperfusion, mean myocardial oxygen consumption was higher in the control group compared with diazoxide (4.49 versus 3.03; $P=0.03$) (Tables S1 through S3). When comparing percentage change from baseline, the difference between groups was not significant (27% versus 39%; $P=0.51$) (Figure 5).

Left Ventricular Ejection Fraction

Mean LVEF was not different between groups at baseline. After 30 minutes of reperfusion, LVEF was 27% higher in the diazoxide group compared with the control ($42.5\% \pm 16.05$ and $15.8\% \pm 12.42$, respectively; $P=0.009$). By 60 minutes of reperfusion, there was no statistically significant difference in LVEF between groups (Figure 6). There were no statistically significant differences between groups in other transthoracic echocardiography measured parameters (Table S2).

Left Ventricular Pressures

Because of catheter malpositioning, only 5 animals in the diazoxide group had 30- and 60-minute data, and only 4 animals in the control group had measurements

at 60 minutes of reperfusion. LVDP, dp/dt min, and dp/dt max were not different between groups at baseline. There were no significant differences between groups in the average dp/dt max, dp/dt min, and LVDP at any time point using Student's t tests (Figure 7).

Linear mixed regression modeling demonstrated that animals treated with diazoxide had 1026 mmHg greater dp/dt max and 24 mmHg greater LVDP after 30 minutes of reperfusion ($P=0.001$ and 0.008 , respectively) compared with controls. There were no significant differences between groups in dp/dt min.

Cardiac Enzymes, Pro-Apoptotic Markers, and Infarct Size Following 30 Minutes of Regional Ischemia and Subsequent 2 Hours of Global Ischemia

There were no differences in the number of apoptotic cells stained with caspase-3 between groups at baseline or after reperfusion (Table S3). Mean myoglobin and troponin I levels were within a normal range in both groups at all time points and were not significantly different between groups (Table S3).

Infarct Size

Left ventricular weight at the end of the experiment was not different between groups, with a mean weight of 139.85 g in the diazoxide group and 139.65 g in the control group ($P=0.98$). Infarct size was not different between groups (diazoxide, 97.11%; control, 97.70%; $P=0.83$) (Figure 8).

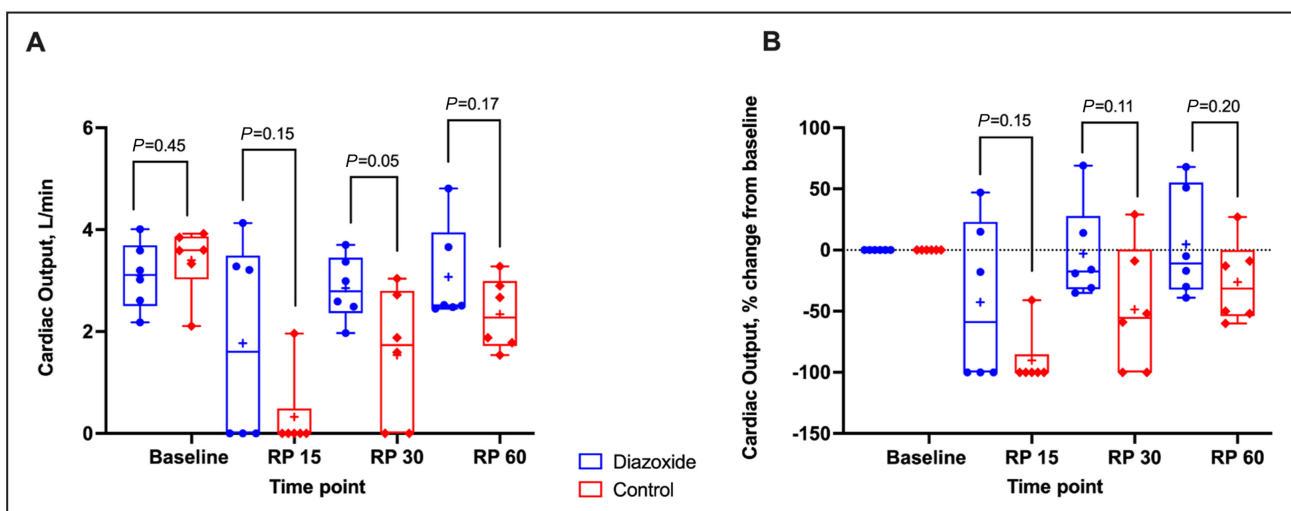


Figure 4. Cardiac output and percent change in cardiac output throughout reperfusion in a porcine model of 30 minutes of regional ischemia, followed by 2 hours of global ischemia.

Animals were randomly assigned to a control group of hyperkalemic cardioplegia alone (red, N=6) or cardioplegia with diazoxide (blue, N=6). Each dot represents an individual animal. The lower and upper borders of each box represent the lower and upper quartile, respectively. The middle line represents the median, and the cross represents the mean. Cardiac output was calculated using the Fick principle at baseline, before regional and global ischemia, and throughout 1 hour of reperfusion. There were no significant differences in mean cardiac output between groups using Student's t test. **A**, Cardiac output represented in liters per minute. **B**, Cardiac output represented as percent change from baseline. RP indicates reperfusion.

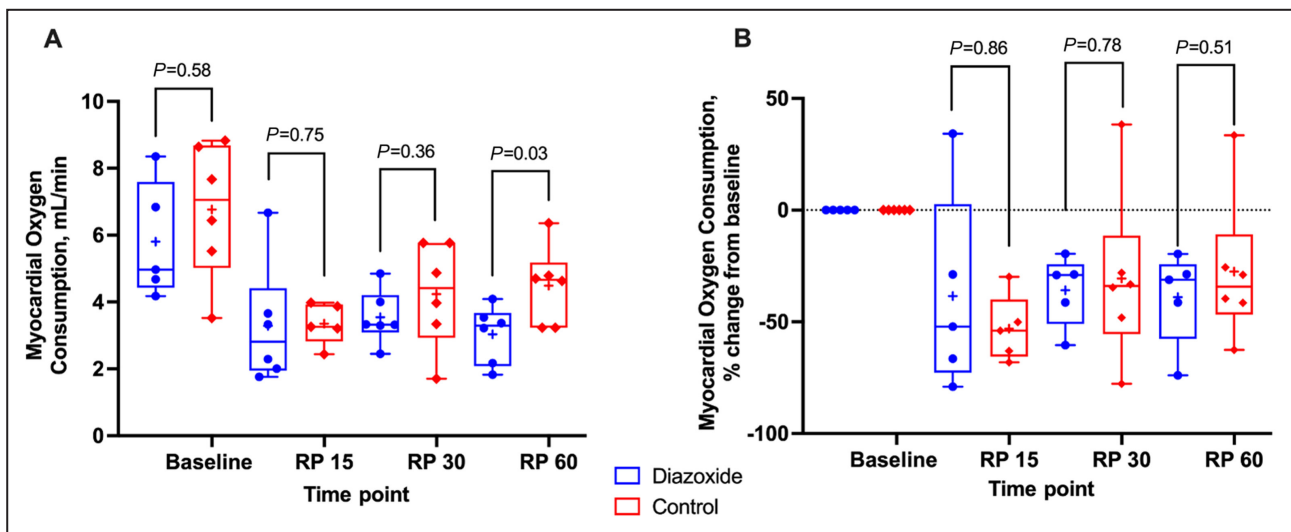


Figure 5. Myocardial oxygen consumption was significantly higher in the control cardioplegia group after 60 minutes of reperfusion in a porcine model of 30 minutes of regional ischemia, followed by 2 hours of global ischemia.

Animals were randomly assigned to a control group of hyperkalemic cardioplegia alone (red, N=6) or cardioplegia with diazoxide (blue, N=6). Each dot represents an individual animal. The lower and upper borders of each box represent the lower and upper quartile, respectively. The middle line represents the median, and the cross represents the mean. Myocardial oxygen consumption was calculated using the Fick principle at baseline, before localized and global ischemia, and throughout 1 hour of reperfusion. Statistical comparisons using Student's *t* test. **A**, Myocardial oxygen consumption represented in mL/min. **B**, Myocardial oxygen consumption represented as percent change from baseline. RP indicates reperfusion.

DISCUSSION

Cardiac surgery is performed with excellent outcomes despite the routine use of hypothermia and electromechanical arrest to provide a quiet and bloodless field. This type of arrest remains the best method known to reduce myocardial oxygen consumption, and it is accomplished using a diverse range of solutions to protect the myocardium during a global ischemic period.²⁰ Unfortunately, cardiac surgery inflicts this global ischemic episode on an already compromised myocardium. In addition, exposure to hypothermic, hyperkalemic cardioplegia provides an additional insult, as it is known to result in myocyte swelling and reduced contractility *in vitro*.^{1,15} It is not surprising that the majority of patients suffer from myocardial stunning after cardiac surgery.^{21,22} Such stunning results in the need for inotropic and mechanical circulatory support, and novel strategies to reduce myocardial stunning are needed. Encouraged by our previous results using K_{ATP} channel opener diazoxide to reduce myocardial stunning in a porcine model of prolonged global ischemia,¹³ we hypothesized that diazoxide would reduce myocardial stunning in a porcine model designed to mimic the situation of a patient undergoing cardiac surgery with ongoing myocardial ischemia.

Using a model of 30 minutes of regional (LAD occlusion) followed by 120 minutes of global ischemia, we found no differences in need for defibrillation after cross-clamp removal or the level of inotropic drugs

needed to wean from CPB between the control group (hypothermic hyperkalemic cardioplegia) and the diazoxide group. However, with equivalent inotropic medications, the diazoxide group had higher LVEF, LVDP, and dp/dt max at 30 minutes of reperfusion compared with the control group, suggesting reduced myocardial stunning. The control group had higher myocardial oxygen consumption at 60 minutes, which may be attributable to increased oxygen extraction, although this requires further study.

There were no differences in myoglobin, troponin, pro-apoptotic markers, or infarct size between groups, likely because of the excellent myocardial protection provided by both groups and because both groups underwent the same regional ischemic episode (LAD occlusion) before CPB and cardioplegia administration. In addition, peak myoglobin and troponin may not have occurred by 60 minutes of reperfusion.

Others have demonstrated that the addition of K_{ATP} channel opener diazoxide (50 μ mol/L) resulted in reduced regional necrosis and apoptosis and mitochondrial damage using a porcine model of acute infarction followed by 30 minutes of global ischemia.²³ Interestingly, the addition of diazoxide had no effect on systolic and diastolic function. We propose that this is attributable to the smaller dose of diazoxide (50 μ M) and the shorter global ischemic period (30 minutes) that is not clinically relevant. In a prolonged global ischemia mouse model, we demonstrated superior diastolic protection with hypothermic hyperkalemic

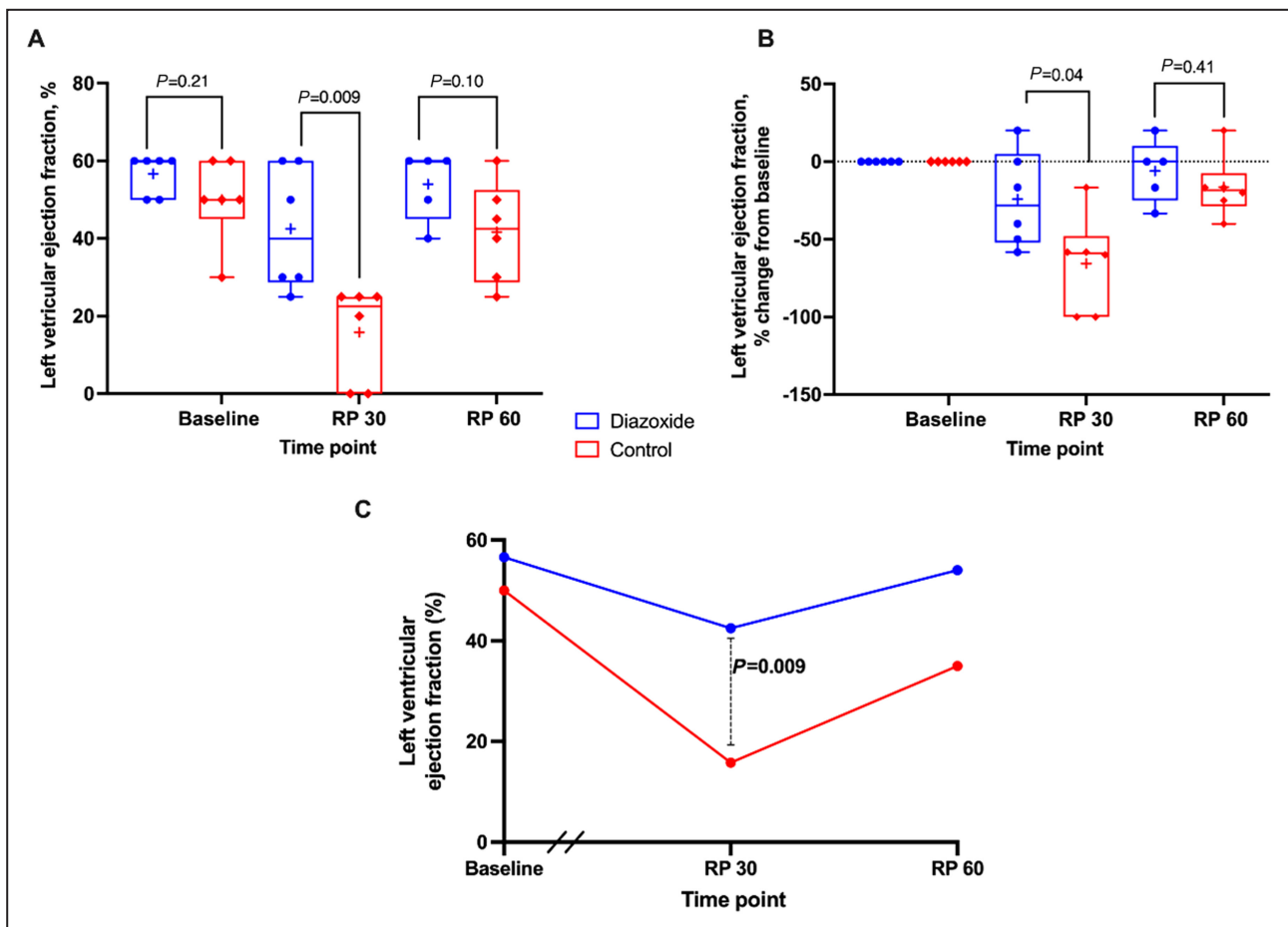


Figure 6. Diazoxide was associated with improved LVEF at 30 minutes of reperfusion in a porcine model of 30 minutes regional ischemia followed by 2 hours global ischemia.

Using a porcine model of 30 minutes of regional ischemia, followed by 2 hours of global ischemia, animals were randomly assigned to a control group of hyperkalemic cardioplegia alone (red, N=6) or cardioplegia with diazoxide (blue, N=6). Each dot represents an individual animal (N=6 in each group). The lower and upper borders of each box represent the lower and upper quartile, respectively. The middle line represents the median, and the cross represents the mean. Left ventricular ejection fraction was measured at baseline, before regional and global ischemia, and throughout 1 hour of reperfusion by transesophageal echocardiography. Statistical comparisons using Student's *t* test. **A**, Left ventricular ejection fraction represented as a percentage. **B**, Left ventricular ejection fraction represented as percent change from baseline. **C**, LVEF represented over time. LVEF indicates left ventricular ejection fraction; and RP, reperfusion.

cardioplegia with diazoxide compared with cardioplegia alone.¹² In humans, diazoxide demonstrated improved cardiac function and reduced markers of injury compared with controls when given as a preconditioning agent before CPB (not as a component of cardioplegia)²⁴ or as a component of warm cardioplegia, in 2 small preliminary human trials of otherwise healthy patients with normal LVEF, no concomitant diseases, and only stable angina.²⁵ However, diazoxide has not been seriously considered as an additive to hypothermic cardioplegia combined with prolonged global ischemia in humans. To provide preclinical data, we designed this clinically relevant porcine model to inform future potential trials in humans. These results suggest that diazoxide may represent a potential pharmacologic method to reduce myocardial stunning after

a regional ischemic episode with a subsequent global ischemic period. The exploitation of diazoxide or other K_{ATP} channel openers to reduce myocardial stunning following cardiac surgery could provide significant impact as hypothermic hyperkalemic cardioplegia is widely used, patients undergoing cardiac surgery are now older and sicker, and surgeries are more complex and associated with longer global ischemia times.^{26–31}

Study Limitations

There are several limitations to this study. This study used healthy, young swine with no known comorbidities in contrast with patients undergoing coronary artery bypass grafting who are more likely to be older and have severe coronary artery disease and other comorbidities. Despite the fact that many patients undergo percutaneous

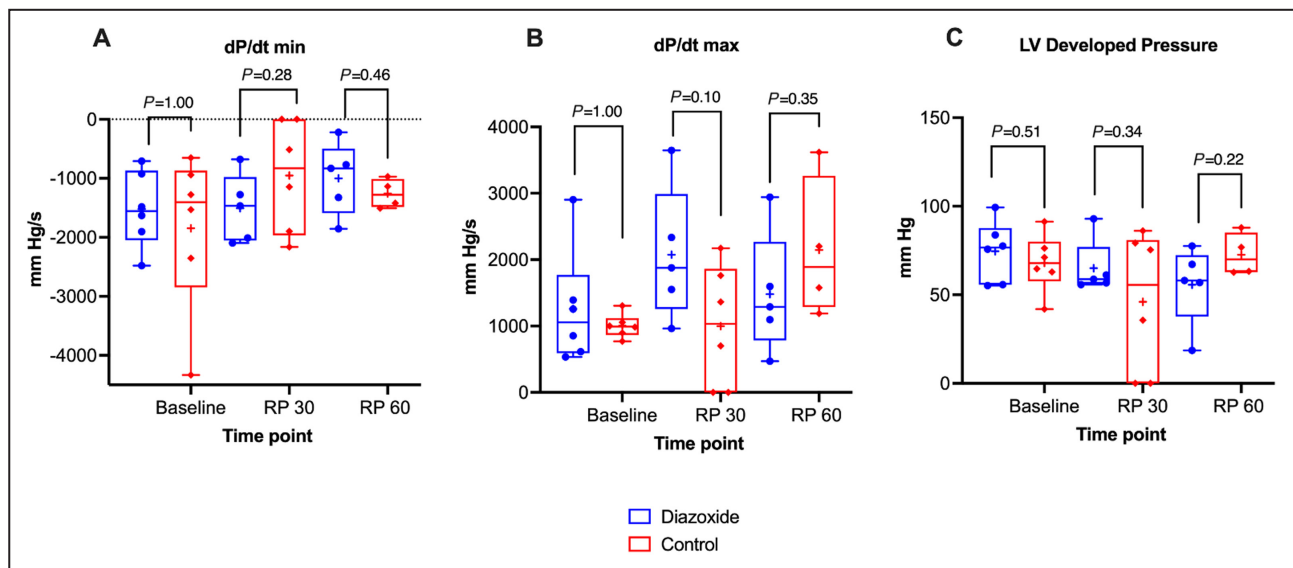


Figure 7. Left ventricular pressures were similar between groups in a porcine model of 30 minutes of regional ischemia, followed by 2 hours of global ischemia.

Animals were randomly assigned to a control group of hyperkalemic cardioplegia alone (red, N=6) or cardioplegia with diazoxide (blue, N=6). A pressure catheter was inserted into the left ventricle, and pressure was monitored at baseline and throughout reperfusion. The lower and upper borders of each box represent the lower and upper quartile, respectively. The middle line represents the median, and the cross represents the mean. Statistical comparisons using Student's *t*-test. **A**, dp/dt min, maximum change in ventricular pressure during isovolumetric relaxation. **B**, dp/dt max, maximum change in ventricular pressure during isovolumetric contraction. **C**, left ventricular developed pressure, calculated as the difference between left ventricular systolic pressure and left ventricular diastolic pressure. LV indicates left ventricle; and RP, reperfusion.

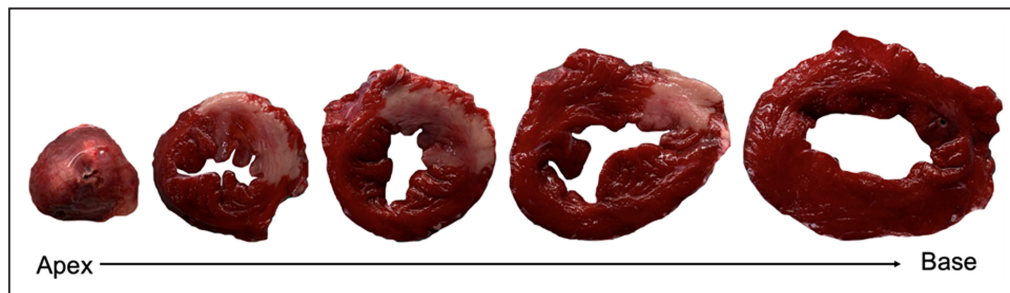


Figure 8. Infarct size in representative sections from apex to base of a heart in the diazoxide group.

In a porcine model of 30 minutes of regional ischemia, followed by 2 hours of global ischemia, animals were randomly assigned to a control group of hyperkalemic cardioplegia alone (N=6) or cardioplegia with diazoxide (N=6). Hearts underwent 30 minutes left anterior descending coronary artery occlusion followed by 2 hours of global ischemia. Upon completion of each experiment, cardiectomy was performed and the left ventricle was sliced into 5 sections. The heart was submerged in a tetrazolium chloride buffer for 15 minutes for myocardial viability assessment. Infarct is visible in the anterior wall, consistent with left anterior descending occlusion. Infarct size was not different between groups.

intervention for acute ischemia before coronary artery bypass grafting, the current model was created to evaluate the effect of diazoxide administered in a clinically relevant method after a regional ischemic episode (an induction dose of diazoxide in hypothermic, hyperkalemic cardioplegia, followed by subsequent cardioplegia doses without diazoxide, during a long ischemic period). Finally, while the sample size and power calculation is consistent with data from previous swine studies and large animal studies from our laboratory, the study is likely underpowered because of the small number.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S3

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Supplemental Material

Table S1. Cardiac output and myocardial oxygen consumption in porcine model of 30 minutes regional ischemia followed by 2 hours global ischemia.

Measurement	Diazoxide, n=6	Control, n=6	P-value
Cardiac output, L/min, mean \pm SD			
Baseline	3.10 \pm 0.66	3.40 \pm 0.67	0.452
RP 15	1.77 \pm 1.97	0.33 \pm 0.80	0.153
RP 30	2.85 \pm 0.63	1.54 \pm 1.30	0.050
RP 60	3.07 \pm 0.97	2.34 \pm 0.70	0.166
Myocardial O ₂ consumption, mean \pm SD			
Baseline	5.81 \pm 1.74	6.77 \pm 2.04	0.584
RP 15	3.29 \pm 1.82	3.35 \pm 0.62	0.748
RP 30	3.54 \pm 0.81	4.24 \pm 1.57	0.356
RP 60	3.03 \pm 0.86	4.49 \pm 1.17	0.034

RP, reperfusion. P value indicates comparison between DZX and control using Student's t-test result.

Table S2. Transeosophageal echocardiogram parameters at baseline, after 30 and 60 minutes of reperfusion.

Measurement	Diazoxide (N=6)	Control (N=6)	P-value
Mitral E velocity, cm/s			
Baseline	46.33 ± 10.93	45.00 ± 10.37	0.833
RP 30	40.00 ± 14.21*	36.50 ± 6.95	0.613
RP 60	45.00 ± 10.98*	42.60 ± 14.33 [†]	0.791
Mitral A velocity, cm/s			
Baseline	43.44 ± 7.31	36.67 ± 4.63	0.089
RP 30	34.75 ± 10.66*	34.50 ± 5.68	0.962
RP 60	38.00 ± 8.52*	40.60 ± 12.58 [†]	0.736
Mitral A duration, ms			
Baseline	105.33 ± 22.50	94.33 ± 15.16	0.344
RP 30	95.40 ± 7.73 [†]	106.17 ± 18.17	0.251
RP 60	104.00 ± 8.72*	106.80 ± 13.63 [†]	0.734
Mitral deceleration time, ms			
Baseline	167.00 ± 51.55	154.00 ± 50.96	0.670
RP 30	142.20 ± 37.96 [†]	157.33 ± 71.02	0.680
RP 60	152.50 ± 31.27*	141.40 ± 23.60 [†]	0.561
Septal e' velocity, cm/s			

Baseline	$4.50 \pm 1.30^*$	5.0 ± 1.41	0.587
RP 30	$6.00 \pm 1.41^*$	4.83 ± 2.40	0.412
RP 60	$5.00 \pm 1.15^*$	4.33 ± 1.75	0.525
Lateral e' velocity, cm/s			
Baseline	5.17 ± 1.47	4.67 ± 1.36	0.556
RP 30	4.67 ± 1.21	5.33 ± 1.97	0.496
RP 60	$4.20 \pm 0.45^\dagger$	5.33 ± 2.34	0.317
Left atrium dimension, cm			
Baseline	2.87 ± 0.39	2.83 ± 0.23	0.859
RP 30	2.78 ± 0.49	2.73 ± 0.35	0.844
RP 60	$2.66 \pm 0.62^\dagger$	2.48 ± 0.39	0.576
LVEF, %			
Baseline	56.67 ± 5.16	50.00 ± 10.95	0.212
RP 30	42.50 ± 16.04	15.83 ± 12.42	0.009
RP 60	54.00 ± 8.94	$41.67 \pm 12.91^\dagger$	0.106
Right heart dimension, cm			
Baseline	2.98 ± 0.33	2.96 ± 0.38	0.937
RP 30	2.98 ± 0.48	$3.08 \pm 0.35^\dagger$	0.715
RP 60	$3.28 \pm 0.62^\dagger$	$3.12 \pm 0.16^\dagger$	0.593

Isovolumetric relaxation time, ms

Baseline	100.00 ± 25.36	93.50 ± 6.77	0.558
RP 30	113.17 ± 25.42	101 ± 18.87	0.369
RP 60	100.80 ± 7.47 [†]	87.00 ± 23.82	0.305

All values represent the mean ± SD. *N=4; [†]N=5; RP, reperfusion; LVEF, left ventricular ejection fraction. P value indicates comparison between DZX and control using Student's t-test result.

Table S3. Serum cardiac enzymes and apoptotic cell count in porcine model of 30 minutes regional ischemia followed by 2 hours global ischemia.

Measurements	Diazoxide, n=6	Control, n=6	P-value
Myoglobin, ng/mL, mean \pm SD			
Baseline	18.36 \pm 6.20	26.28 \pm 10.36	0.140
RP 15	12.94 \pm 11.85	15.21 \pm 8.30	0.718
RP 30	9.83 \pm 7.88	13.12 \pm 7.36	0.473
RP 60	8.70 \pm 6.81	12.36 \pm 5.29	0.320
Troponin I, pg/mL, mean \pm SD			
Baseline	47.33 \pm 6.56	52.33 \pm 10.31	0.339
RP 15	50.56 \pm 8.27	64.17 \pm 35.88	0.431
RP 30	52.47 \pm 18.32	50.63 \pm 12.88	0.844
RP 60	47.21 \pm 12.56	50.78 \pm 9.91	0.597
Caspase 3, number of cells, mean \pm SD			
Baseline	40.40 \pm 30.77	43.10 \pm 28.88	0.881
RP 60- Anterior wall distal to LAD occlusion	44.10 \pm 52.07	51.17 \pm 46.40	0.809
RP 60- Anterior wall proximal to LAD occlusion wall	54.20 \pm 48.07	59.00 \pm 50.36	0.869

RP, reperfusion, LAD, left anterior descending. P value indicates comparison between DZX and control using Student's t-test result.