Diazoxide protects myocardial mitochondria, metabolism, and function during cardiac surgery: A double-blind randomized feasibility study of diazoxide-supplemented cardioplegia

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Objectives: The study was designed to assess whether diazoxide-mediated cardioprotection might be used in human subjects during cardiac surgery.

Methods: Forty patients undergoing coronary artery bypass grafting were randomized to receive intermittent warm blood antegrade cardioplegia supplemented with either diazoxide (100 $\mu\text{mol/L}$) or placebo ($n = 20$ in each group). Mitochondria were assessed before and after ischemia and reperfusion in myocardial biopsy specimens. Myocardial oxygen and glucose and lactic acid extraction ratios were measured before ischemia and in the first 20 minutes of reperfusion. Hemodynamic data were collected, and troponin I, creatine kinase-MB, and N-terminal prohormone brain natriuretic peptide levels were measured. All outcomes were analyzed by using mixed-effects modeling for repeated measures.

Results: No deaths, strokes, or infarcts were observed. Patients received, on average, 36.2 ± 1.2 mg of diazoxide and 37.3 ± 1.9 mg of placebo ($P = .6$). Diazoxide added to cardioplegia prevented mitochondrial swelling (8899 ± 474 vs 9273 ± 688 pixels before and after the procedure, respectively; $P = .6$) compared with that seen in the placebo group (8474 ± 163 vs $11,357 \pm 759$ pixels, $P = .004$). No oxygen debt was observed in the diazoxide group. Glucose consumption and lactic acid production returned to preischemic values faster in the diazoxide group. The following hemodynamic parameters differed between the diazoxide and placebo groups, respectively, in the postoperative period: cardiac index, 3.0 ± 0.09 versus 2.6 ± 0.09 $\text{L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ ($P = .002$); left cardiac work index, 2.81 ± 0.07 versus 2.31 ± 0.07 kg/m^2 ($P < .001$); oxygen delivery index, 420 ± 14 versus 377 ± 13 $\text{mL} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$ ($P = .03$); and oxygen extraction ratio, $29.3\% \pm 1.1\%$ versus $32.6\% \pm 1.1\%$ ($P = .02$). Postoperative myocardial enzyme levels did not differ, but N-terminal prohormone brain natriuretic peptide levels were lower in the diazoxide group (120 ± 27 vs 192 ± 29 pg/mL , $P = .04$).

Conclusions: Supplementing blood cardioplegia with diazoxide is safe and improves myocardial protection during cardiac surgery, possibly through its influence on the mitochondria.

Supplemental material is available online.

There is substantial evidence from in vitro and experimental animal studies that diazoxide provides a strong myocardio-protective effect.¹ Diazoxide is a selective mitochondrial adenosine triphosphate-sensitive potassium (K_{ATP}) channel opener and is believed to induce a preconditioning state.¹

Opening mitochondrial K_{ATP} channels leads to (microscopically undetectable) mitochondrial volume increase, which results in tighter apposition of specialized sites on internal and external mitochondrial membranes associated with coupling to cytosol creatine kinase.² This allows for fatty acid transport into the mitochondrion³ and speeds up oxidative phosphorylation.⁴ Simultaneously, diazoxide prevents the “priming phase” of mitochondrial permeability transition pore opening and thus pathologic mitochondrial swelling, leading to eventual cell death.⁵ Recently, it has also been postulated that diazoxide might exert its cardioprotective action during ischemia through intracellular targets other than mitochondrial K_{ATP} channels.^{6,7} We have shown in human atrial trabeculae that administering diazoxide at the relatively high concentration of 100 $\mu\text{mol/L}$ throughout ischemia provides a significantly stronger cardioprotective effect.^{8,9}

Diazoxide has never been implemented in human studies, apart from the trial in which it was administered systemically as a preconditioning signal before cardiopulmonary bypass (CPB).¹⁰ The present human study was designed to assess

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Abbreviations and Acronyms

AXC	= aortic crossclamping
CK-MB	= creatine kinase-MB
CPB	= cardiopulmonary bypass
K _{ATP}	= adenosine triphosphate-sensitive potassium
NT-proBNP	= N-terminal prohormone brain natriuretic peptide
O ₂ ER	= oxygen extraction ratio

whether supplementing blood cardioplegia with 100 $\mu\text{mol/L}$ diazoxide is safe and confers a beneficial effect on myocardial mitochondria,^{5,11,12} metabolism,^{11,13,14} function,^{8,9,15,16} and viability^{8,9,16,17} after ischemia and reperfusion, as suggested by prior experimental in vitro and animal studies.

MATERIALS AND METHODS

The Medical University of Silesia Bioethics Committee approved the study, and informed consent was obtained from all participating patients. The study was performed on patients undergoing elective coronary artery bypass grafting for triple-vessel coronary artery disease and stable angina. Exclusion criteria included acute coronary syndrome, left ventricular ejection fraction of less than 35%, coexisting valvular disease of at least moderate severity, coexisting illnesses that might influence perioperative risk, and diabetes mellitus. We evaluated 162 patients over the time period of 13 months and identified 63 eligible patients, of whom 41 agreed to randomization. One patient was excluded because during the procedure, technical difficulties forced us to reperform the anastomosis. The patient was found to be in the placebo group once the study was unblinded. Intermittent warm blood (37°C) antegrade cardioplegia was used for myocardial protection. The patients were randomized based on a random-digit table to receive 100 $\mu\text{mol/L}$ diazoxide or placebo as a cardioplegia supplement. Oxygenated blood was infused through an aortic root needle at a rate of 300 mL/min. By using a syringe pump, potassium chloride was added to keep the potassium concentration at 20 mEq/L at induction and 10 mEq/L during maintenance. Simultaneously, another syringe pump was used to supplement cardioplegia with diazoxide (Eudemine Injection; Goldshield Pharmaceuticals Ltd, Croydon, United Kingdom) to achieve a concentration of 100 $\mu\text{mol/L}$ or to infuse placebo (0.9% sodium chloride). Neither the perfusionist, the anesthetist, nor the surgeon knew the content of the syringe. Each patient was administered a 3-minute cardioplegia infusion (containing 21 mg of diazoxide/placebo) at induction and a 1.5-minute infusion as a maintenance dose (containing 10 mg of diazoxide/placebo) every 12 to 15 minutes. Anesthesia consisted of 15 mg of midazolam administered orally 1 hour before surgical intervention; 0.2 mg/kg etomidate, 5 $\mu\text{g/kg}$ fentanyl, and 0.1 mg/kg pancuronium administered intravenously for anesthesia induction; and 0.1 mg $\cdot$ kg⁻¹ $\cdot$ h⁻¹ midazolam and 6 $\mu\text{g} \cdot$ kg⁻¹ $\cdot$ h⁻¹ fentanyl infusion for anesthesia maintenance. No anesthetic gases were used.

Hemodynamic Status

A pulmonary artery catheter was used to assess the hemodynamic profile and the patient's oxygen metabolism status preoperatively and within the first postoperative 24 hours (see Appendix E1). During CPB, mean arterial pressure, central venous pressure, and systemic vascular resistance index were acquired before aortic crossclamping (AXC), immediately after every dose of cardioplegia, 2 minutes later, and immediately before every next dose of cardioplegia. Measurements were also obtained before removing the AXC and before weaning the patient off CPB. Mean arterial pressure

was kept between 50 and 80 mm Hg by using glyceryl trinitrate infusion and norepinephrine 1:100,000 1-mL boluses. All patients were weaned off CPB by using 5 $\mu\text{g} \cdot$ kg⁻¹ $\cdot$ min⁻¹ dopamine infusion and 0.25 $\mu\text{g} \cdot$ kg⁻¹ $\cdot$ min⁻¹ glyceryl trinitrate, which are routinely used in our institution to improve visceral perfusion and diuresis. No inotropic drugs were administered during the first 20 minutes of reperfusion. After transferring the patient to the intensive care unit, the rate of infusions was adjusted according to patient hemodynamic and clinical status by the team on call, who were unaware of the myocardial protection used.

Myocardial metabolism. Measurements of serum glucose (Biosystem, Barcelona, Spain), lactic acid (Randox Laboratories Ltd, Crumlin, United Kingdom), and blood gases (RapidLab 865 blood gas analyzer; Bayer Diagnostics, Warszawa, Poland) were performed on blood samples drawn from the aortic root and from the coronary sinus before AXC, immediately after removing the AXC, and 10 and 20 minutes later. Myocardial oxygen, glucose, and lactic acid extraction ratios were calculated (see Appendix E1).

Myocardial mitochondria. True-cut needle biopsy specimens obtained by using a 16-gauge needle were taken from the apical left ventricle before AXC and 1 hour after AXC and placed in cacodyl buffer with 2% glutaraldehyde. Glutaraldehyde-fixed samples were postfixed with 1% osmium, dehydrated with ethanol, fixed with propylene oxide, and then embedded in epoxy resin. After polymerization in increasing temperatures, semithin sections were obtained with an ultramicrotome (Reichert, Vienna, Austria) and stained with toluidine blue. Ultrathin sections were placed on copper grids and stained with uranyl acetate and lead citrate. The mitochondria were micrographed with a JEOL-JEM 100CX transmission electron microscope (JEOL, Inc, Peabody, Mass) at 16,000 $\times$ magnification. The electron micrographs were next saved at 300-dpi resolution, and the median surface area in pixels of all mitochondria with the outline completely within the photograph was established on 3 photographs from every sample. On average, 15.4 $\pm$ 6.8 mitochondria were measured per photograph.

Myocardial enzymes. Troponin I (immunoenzymatic method; Bio-merieux, Lyon, France) and creatine kinase-MB (CK-MB; immunokinetic method; Thermo Electron Co, Altrincham, United Kingdom) levels were measured before the operation and 6, 24, and 48 hours after removing the AXC. N-terminal prohormone brain natriuretic peptide (NT-proBNP) plasma levels were measured before the operation, 24 hours after removing the AXC, and 5 days postoperatively (immunoenzymatic method; Biomedica, Bratislava, Slovakia).

Patient Characteristics

Mean patient age was 61 $\pm$ 8 and 64 $\pm$ 7 years ($P = .2$) in the diazoxide and placebo groups, respectively. There were 15 and 12 ($P = .5$) female patients in each group, respectively. Mean Canadian Cardiovascular Society class was 2.6 $\pm$ 0.7 and 2.4 $\pm$ 0.8 ($P = .6$), and ejection fraction was 51% $\pm$ 5% and 50% $\pm$ 5% ($P = .5$), respectively. No statistically significant differences were found in clinical characteristics between the groups (see Table E1).

In all patients the left internal thoracic artery was anastomosed to the left anterior descending artery, and 2 or 3 saphenous vein grafts were constructed. The mean number of distal anastomoses was 3.1 $\pm$ 0.2 in the diazoxide group and 3.3 $\pm$ 0.7 in the placebo group ($P = .2$). AXC time was 41 $\pm$ 9 and 45 $\pm$ 9 minutes ($P = .1$), and CPB time was 79 $\pm$ 9 and 86 $\pm$ 18 minutes ($P = .2$), respectively.

Statistical Methods

Preoperative data are presented as means $\pm$ standard deviation, and postoperative outcomes are presented as means $\pm$ standard error of the mean. Mixed-effects modeling for repeated measures, with protection type as a fixed factor and time of measurement as random factor, was used to compare the time course of different parameters. Preoperative parameter values

TABLE 1. Hemodynamics during cardiopulmonary bypass

	MAP (mm Hg)		SVRI (dynes · s ⁻¹ · m ⁻² · cm ⁻⁵)		CVP (mm Hg)	
	Diazoxide group	Placebo group	Diazoxide group	Placebo group	Diazoxide group	Placebo group
Before AXC	60 ± 4	62 ± 3	1948 ± 123	1981 ± 104	2 ± 1	2 ± 1
After cardioplegia	32 ± 2	34 ± 2	1192 ± 93	1173 ± 67	2 ± 1	2 ± 1
2 Min after	50 ± 5	48 ± 3	1692 ± 173	1556 ± 98	3 ± 1	3 ± 1
Before cardioplegia	62 ± 4	60 ± 3	2028 ± 150	1988 ± 102	2 ± 1	1 ± 1
After cardioplegia	39 ± 4	36 ± 3	1446 ± 130	1368 ± 120	2 ± 1	3 ± 1
2 Min after	51 ± 4	50 ± 3	1694 ± 130	1661 ± 127	1 ± 2	2 ± 1
End of AXC	59 ± 4	63 ± 3	2002 ± 138	2050 ± 118	1 ± 1	1 ± 1
End of CPB	61 ± 3	88 ± 24	1868 ± 109	3050 ± 915	1 ± 3	3 ± 1

All data are presented as means ± standard error of the mean. Diazoxide group, n = 20; placebo group, n = 20. Mixed-effects modeling with protection type as fixed and time as random factor was used (systemic vascular resistance index: protection, $P = .9$; mean arterial pressure: protection, $P = .4$; central venous pressure: protection, $P = .4$). MAP, Mean arterial pressure; SVRI, systemic vascular resistance index; CVP, central venous pressure; AXC, aortic crossclamp; CPB, cardiopulmonary bypass.

were included in the analysis as covariates with random effect. Where a single value for the entire evaluation period is given, it is a marginal mean estimated from the mixed-effects model. The same model was used to estimate the mean difference between the groups. Patient characteristics and outcome data at a given time point were compared by using the t test. Qualitative data were compared with Fisher's exact test.

The size of mitochondria in biopsy specimens before and after the operation was expressed as the mean ± standard error and compared with 2-way analysis of variance. SPSS for Windows 14.0 (SPSS, Inc, Chicago, Ill) was used for statistical analysis.

RESULTS

Intraoperative Course

The diazoxide group received a total of 1575 ± 52 mL and the placebo group received a total of 1620 ± 83 mL of cardioplegia ($P = .6$), which corresponded to 36.2 ± 1.2 mg of diazoxide and 37.3 ± 1.9 mg of placebo ($P = .6$). No differences in systemic vascular resistance index changes and mean arterial pressure changes were observed between groups (Table 1). During CPB, intervention to increase arterial pressure was necessary in 16 patients in the diazoxide group and 14 patients in the placebo group ($P = .7$). On average, 5.5 ± 1.5 and 4.6 ± 1.3 mL of 1:100,000 norepinephrine per patient was administered in the diazoxide and placebo groups, respectively ($P = .6$). Intervention to lower arterial pressure was undertaken in 6 patients receiving diazoxide and 2 patients receiving placebo ($P = .2$).

Two patients in the diazoxide group and 3 in the placebo group ($P = 1.0$) required DC shock after declamping the aorta. All others regained sinus rhythm spontaneously.

Myocardial Metabolism

We observed significant differences in myocardial metabolism between the groups in the early phase of reperfusion (Figure 1). The differences were most pronounced 10 minutes after removing the AXC.

At 10 minutes of reperfusion, the myocardial oxygen extraction ratio (O₂ER) was significantly higher in comparison with the preischemic value in the placebo group ($53\% \pm 3\%$ vs $47\% \pm 3\%$, $P = .02$) but did not differ in patients

treated with diazoxide ($44\% \pm 3\%$ vs $46\% \pm 3\%$, $P = .2$); there was a significant difference between the groups ($P = .009$). After 20 minutes of reperfusion, the myocardial O₂ER was practically back to preischemic values in both groups. In a mixed-effects model the myocardial O₂ER in reperfusion was significantly higher in the placebo group ($P = .02$; Figure 1, A).

Myocardial glucose consumption in reperfusion assessed with the glucose extraction ratio was lower in the diazoxide group ($P = .03$; Figure 1, B). Glucose extraction was very high immediately after AXC and decreased over 20 minutes of reperfusion to almost preischemic levels. Characteristically, at 10 minutes of reperfusion, the glucose extraction ratio was significantly above the preischemic level only in the placebo group ($P = .001$), and at this time point, a difference between the 2 groups was observed ($P = .03$).

Lactic acid production by the myocardium resulted in a negative lactate extraction ratio. It returned to preischemic values within 10 minutes of reperfusion in the diazoxide-treated group but required 20 minutes of reperfusion to normalize in the placebo group (Figure 1, C). Myocardial lactic acid production was significantly higher 10 minutes after AXC in the placebo group than in the diazoxide group ($-55\% \pm 14\%$ vs $-24\% \pm 6\%$, respectively; $P = .04$).

Mitochondria

The size of mitochondria in the left ventricular myocardium increased during ischemia and reperfusion, from 8474 ± 163 pixels to $11,357 \pm 759$ pixels ($P = .004$) in the placebo group, whereas diazoxide prevented ischemia and reperfusion-induced mitochondrial swelling (8899 ± 474 vs 9273 ± 688 pixels before and after the procedure, respectively; $P = .6$). The different influence of time on mitochondrial size in the 2 groups was confirmed by means of 2-way analysis of variance ($P = .03$, Figure 2).

Postoperative Course

No death, perioperative myocardial infarction, or stroke occurred. One re-exploration for bleeding was necessary in

the diazoxide group, and 1 postoperative delirium occurred in the placebo group. Patients were ventilated for 15.6 ± 0.9 hours in the diazoxide group and 19.5 ± 1.0 hours in the placebo group ($P = .007$).

All patients were weaned off CPB with the help of infusion of $5 \mu g \cdot kg^{-1} \cdot min^{-1}$ dopamine. No other inotropes or intra-aortic balloon counterpulsation was necessary. Dopamine infusion was gradually weaned off within 24 hours in the diazoxide group, whereas its maintenance appeared necessary in the placebo group ($P = .005$). One day after AXC, diazoxide group patients were receiving a mean dopamine dose of $1.6 \pm 0.5 \mu g \cdot kg^{-1} \cdot min^{-1}$, whereas placebo group patients were receiving $4.1 \pm 0.4 \mu g \cdot kg^{-1} \cdot min^{-1}$ ($P < .001$). Even with lower inotropic support in the postoperative period, mean cardiac index was $0.4 \pm 0.1 L \cdot min^{-1} \cdot m^{-2}$ higher ($P = .002$) in the diazoxide group than in the placebo group (Figure 3). Neither central venous pressure (6.6 ± 0.3 vs 6.8 ± 0.3 mm Hg, $P = .7$), pulmonary capillary wedge pressure (8.5 ± 0.3 vs 8.0 ± 0.3 mm Hg, $P = .3$), mean arterial pressure (75 ± 1 vs 72 ± 1 mm Hg, $P = .063$), nor mean pulmonary artery pressure (16.9 ± 0.5 vs 17.1 ± 0.5 mm Hg, $P = .8$) differed between the groups. Postoperative systolic blood pressure was significantly higher in the diazoxide group (113 ± 2 vs 106 ± 2 mm Hg, $P = .02$), whereas systemic vascular resistance index did not change. Left ventricular work index was higher by $0.51 \pm 0.095 kg/m^2$ ($P < .001$) in the diazoxide group postoperatively (Figure 4). Oxygen delivery index was higher by $48 \pm 21 mL \cdot min^{-1} \cdot m^{-2}$ ($P = .02$) in the diazoxide group (see Figure E1), but postoperative oxygen consumption remained similar in both groups (127 ± 3 vs $121 \pm 3 mL \cdot min^{-1} \cdot m^{-2}$, $P = .2$). As a result, the postoperative O_2ER was lower in the diazoxide group ($29.3\% \pm 1.1\%$ vs $32.6\% \pm 1.1\%$, $P = .004$).

Myocardial Enzymes

Neither postoperative troponin I ($P = .3$) nor CK-MB ($P = .3$) levels differed significantly between the groups. The highest postoperative troponin I level occurred at 6 hours after AXC and reached a value of 2.5 ± 0.4 ng/mL in the placebo group and 1.8 ± 0.2 ng/mL in the diazoxide group ($P = .2$). CK-MB levels were highest 24 hours after removing the AXC (53.0 ± 8.5 and 50.4 ± 8.0 IU/L in the placebo and diazoxide groups, respectively; $P = .8$). The NT-proBNP level increased significantly in the postoperative period

FIGURE 1. Myocardial metabolism during the first 20 minutes after aortic cross-clamp (AXC) removal. All data are presented as means $\pm$ standard error of the mean. Mixed-effects modeling with protection type as fixed and time as random factor was used ($n = 20$ for every group). * $P < .05$ between groups at the same time point. A, Oxygen extraction ratio (O_2ER ; protection, $P = .017$). B, Glucose extraction ratio ($Glucose\ ER$; protection, $P = .033$). C, Lactic acid extraction ratio ($Lactate\ ER$; protection, $P = .077$).

FIGURE 2. Electrophotomicrographs of left ventricular myocardial mitochondria from the diazoxide group before (A) and after (B) ischemia and reperfusion and the placebo group before (C) and after (D) ischemia and reperfusion. (Original magnification $\times 16,000$.) The middle panel shows the influence of diazoxide on the mitochondria size (pixels) before (*Pre op*) and after (*Post op*) ischemia and reperfusion. Data are presented as means $\pm$ standard error of the mean. Two-way analysis of variance was used to check the influence of time and protection type on mitochondrial size (protection, $P = .1$; time, $P = .005$; time $\times$ protection, $P = .03$). * $P < .05$ between groups at the same time point.

($P = .001$). Its concentration was, on average, 72 ± 35 pg/mL less in the diazoxide group ($P = .04$, Figure 5).

DISCUSSION

The current study supports the safety of supplementing blood cardioplegia with 100 μ mol/L diazoxide because we did not observe the negative effect on blood pressure that has been reported in a porcine model during reperfusion¹ or with adenosine supplementation.¹⁸ This was not unexpected because an intravenous bolus dose of 1 mg/kg diazoxide causes only around a 10% decrease in blood pressure.¹⁹ Indeed, postoperative systolic blood pressure in this experiment was actually higher in the diazoxide-treated patients, possibly related to a higher cardiac index because all other hemodynamic parameters remained equal. Furthermore, the study corroborates the protective mechanism afforded by diazoxide against ischemic mitochondrial swelling. Diazoxide increases mitochondrial volume, presumably by opening mitochondrial K_{ATP} channels. These subtle changes are undetectable microscopically¹³ but are thought to bring inner and outer mitochondrial membrane enzymes closer and hence facilitate phosphorylation. Conversely, ischemic swelling is caused by increased mitochondrial permeability transition pore opening, which leads to microscopically obvious swelling and eventually rupture of the mitochondrion.⁵ Diazoxide has been shown to significantly reduce this phenomenon in isolated rat mitochondria¹¹ and in an in vivo porcine model.¹² The current data confirm these findings for the first time in clinical settings.

FIGURE 3. Cardiac index (CI) before the operation, before cardiopulmonary bypass (CPB), and within the first 24 hours postoperatively. Data are presented as means $\pm$ standard error of the mean. Mixed-effects modeling with protection type as fixed and time as random factor was used (protection, $P = .002$). * $P < .05$ between groups at the same time point ($n = 20$ for every group).

Our study also confirms the beneficial influence of diazoxide on myocardial metabolism during ischemia and reperfusion. Increased oxygen consumption in reperfusion, as observed 10 minutes after removing the AXC in the placebo group, has long been recognized as a characteristic “oxygen debt,”²⁰ and reduced oxygen consumption during this phase has served as a marker of better myocardial protection.²¹ In

FIGURE 4. Left cardiac work index (LCWI; A) and right cardiac work index (RCWI; B) before the operation, before cardiopulmonary bypass (CPB), and within the first 24 hours postoperatively. Data are presented as means $\pm$ standard error of the mean. Mixed-effects modeling with protection type as fixed and time as random factor was used (left cardiac work index: protection, $P < .001$; right cardiac work index: protection, $P = .1$). * $P < .05$ between groups at the same time point ($n = 20$ for every group).

our study no significant oxygen debt was noted in diazoxide-protected hearts. The mechanism of action is still debatable but might be K_{ATP} channel independent because pinacidil (a nonselective K_{ATP} channel activator)-based cardioplegia in a rabbit model resulted in higher oxygen consumption during reperfusion than potassium cardioplegia.²² Increased glucose consumption and lactic acid production can be considered a sign of still uncoupled glycolysis and glucose oxidation with an inability to metabolize pyruvate in the Krebs cycle.²³ It was shown that diazoxide leads to tighter apposition of specialized sites on internal and external mitochondrial membranes associated with coupling to cytosol creatine kinase² and as a result allows for fatty acid transport into the mitochondrion³ and speeds up oxidative phosphorylation.⁴ The faster recovery of oxidative metabolism with no lactate production observed in the diazoxide group could

FIGURE 5. N-terminal prohormone brain natriuretic peptide (NT-proBNP) levels preoperatively (pre-op) and within 5 days postoperatively. Bars depict means $\pm$ standard error of the mean. Mixed-effects modeling with protection type as fixed and time as random factor was used (protection, $P = .048$; $n = 20$ for every group).

potentially be attributed to this mechanism. Previous reports of increased mitochondrial respiratory rate¹³ and higher adenosine triphosphate levels¹⁴ after ischemia in diazoxide-treated isolated rat hearts further support our data. The positive metabolic effect of diazoxide in our study was most likely caused by direct intracoronary application during ischemia because preoperative intravenous infusion to induce “preconditioning” has failed to show a similar effect.¹⁰ Similarly, in our previous experiments on isolated human myocardium, diazoxide prevented ischemic contracture only when present during ischemia and not as a preconditioning agent.¹⁸ These observations were supported by the findings of other investigators in isolated mitochondria.¹¹

The current study failed to find significant differences in CK-MB and troponin I levels in the postoperative period between the groups. Most experimental studies show that diazoxide significantly protects against ischemia-induced myocardial necrosis.^{1,8,9,15,17} However, many of these tested diazoxide-mediated protection against no protection. In a cardiac surgical rabbit model, potassium/magnesium cardioplegia decreased the size of myocardial infarction after 30 minutes of ischemia from $27.8\% \pm 2.4\%$ to $3.7\% \pm 0.5\%$, and administration of diazoxide further reduced the infarction area to $1.5\% \pm 0.4\%$.¹⁶ Therefore the small but potentially important benefit of diazoxide supplementation on myocardial necrosis might not be reflected in differential myocardial enzyme levels. Similarly low necrosis marker concentration in our relatively “healthy” placebo group undergoing rather uncomplicated and short coronary artery grafting procedures could mask the influence of diazoxide on myocardial cell death.

The positive effect of diazoxide on postoperative cardiac function without reduction of myocardial necrosis suggests

an antistunning effect of diazoxide supplementation. With less inotropic support, diazoxide-treated patients had a significantly higher mean postoperative cardiac index with similar loading conditions. Improved myocardial function was further documented by significantly higher left ventricular cardiac work index, which is often regarded as the best clinical surrogate of contractility.²⁴ It is tempting to conjecture that increased contractility resulted in a significantly higher oxygen delivery index and, as a result, $3\% \pm 1\%$ lower O_2ER in the diazoxide group in the postoperative period. This difference might appear small, but a reduction of mixed venous oxygen saturation by 5% has been described as a predictor of postoperative complications.²⁵

Lower postoperative NT-proBNP levels observed in the diazoxide group might reflect less myocyte stretch resulting from better diastolic or systolic function. Although the predictive value of postoperative NT-proBNP measurement has not been unanimously established, it is likely that postoperative NT-proBNP levels correlate with the probability of cardiovascular events²⁶ and myocardial remodeling after acute myocardial infarction.²⁷ Studies with sevoflurane as a preconditioning agent suggest that lower postoperative NT-proBNP levels reflect better myocardial protection.²⁸

In conclusion, the current study has confirmed the safety of diazoxide supplementation to blood cardioplegia in a selected group of low-risk cardiac surgical patients. We have also demonstrated a positive effect on myocardial metabolism and postoperative function. Further clinical studies are needed to better define the role of diazoxide-based cardioplegia in the surgical armamentarium.

This work represents primarily a safety study, and therefore only a small number of relatively healthy patients with well-preserved left ventricular function undergoing an uncomplicated cardiac procedure were included. Because of this, no attempt has been made to extrapolate clinical endpoints from these data. At the same time, the choice of the study group decreased the chance of finding a positive treatment effect. Not surprisingly, the differences observed (functional and metabolic) were small and transient. We acknowledge that ideally the amount of necrosis and apoptosis should have been assessed directly in biopsy specimens. Having a limited amount of tissue, we decided to concentrate on mitochondrial assessment, but this limitation must be duly acknowledged.

We excluded diabetic patients from the study because sulfonylurea derivatives often used in type 2 diabetes mellitus inhibit K_{ATP} channels and might therefore affect diazoxide's action.²⁹ Many authors also believe that preconditioning mechanisms are impaired in diabetes,¹⁷ and additionally, diazoxide has intrinsic hyperglycemic properties.

Our study group had a high percentage of female patients. It has been recently suggested, based on an animal study, that diazoxide might be less effective in preventing postischemic function loss, myocardial infarction, and mitochon-

drial calcium overload and dysfunction in female subjects, particularly with increasing age.³⁰ Our group is obviously too small to comment on sex differences. However, the distribution of female subjects was equal between the groups, and it is possible that the observed functional and metabolic differences might be magnified in a male population.

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APPENDIX E1. FORMULAE USED FOR CALCULATION OF HEMODYNAMIC AND METABOLIC PARAMETERS

Myocardial metabolism in reperfusion

Parameters measured:

- Hemoglobin concentration (Hgb)
- Arterial partial pressure of oxygen (P_aO_2)
- Arterial hemoglobin oxygen saturation ($SatO_2$)
- Arterial concentration of glucose ($[glucose]_a$)
- Arterial concentration of lactate ($[lactate]_a$)
- Coronary sinus partial pressure of oxygen (P_vO_2)
- Coronary sinus hemoglobin oxygen saturation (Sat_vO_2)
- Coronary sinus concentration of glucose ($[glucose]_v$)
- Coronary sinus concentration of lactate ($[lactate]_v$)

Parameters calculated:

- Arterial oxygen content

$$C_aO_2 = (1.38 \bullet Hgb \bullet SatO_2) - (0.0031 \bullet P_aO_2)$$

- Coronary sinus oxygen content

$$C_vO_2 = (1.38 \bullet Hgb \bullet Sat_vO_2) + (0.0031 \bullet P_vO_2)$$

- Myocardial oxygen extraction ratio

$$O_2ER = \frac{C_aO_2 - C_vO_2}{C_aO_2}$$

- Myocardial glucose extraction ratio

$$GlucoseER = \frac{[glucose]_a - [glucose]_v}{[glucose]_a} \bullet 100$$

- Myocardial lactate extraction ratio

$$LactateER = \frac{[lactate]_a - [lactate]_v}{[lactate]_a} \bullet 100$$

Hemodynamic parameters during cardiopulmonary bypass

Parameters measured:

- Pump output
- Mean arterial pressure (MAP)
- Central venous pressure (CVP)

Parameters calculated:

- Systemic vascular resistance index

$$SVRI = \frac{(MAP - CVP) \bullet BSA}{\text{pump output}} \bullet 80$$

Hemodynamic parameters in first postoperative 24 hours

Parameters measured:

- Cardiac output with thermodilution method

$$CO = \frac{V(T_B - T_I)(S_I \bullet C_I) \bullet 60C_T \bullet K}{A \bullet (S_B \bullet C_B)!}$$

where V = injected volume, A = area under the temperature curve, K = calibration constant, T_B = blood temperature, T_I = temperature of injectate, S_B = blood-specific weight, S_I = injectate-specific weight, C_B = blood-specific heat capacity, S_I = injectate-specific heat capacity, C_T = correction for injectate heating

- Heart rate (HR)
- Mean arterial pressure (MAP)
- Mean pulmonary artery pressure (MPAP)
- Central venous pressure (CVP)
- Pulmonary capillary wedge pressure (PCWP)
- Hemoglobin concentration (Hgb)
- Arterial partial pressure of oxygen (P_aO_2)
- Arterial hemoglobin oxygen saturation ($SatO_2$)
- Mixed venous partial pressure of oxygen ($P_{MV}O_2$)
- Mixed venous hemoglobin oxygen saturation ($Sat_{MV}O_2$)

Parameters calculated:

- Cardiac index

$$CI = \frac{CO}{BSA}$$

- Systemic vascular resistance index

$$SVRI = \frac{MAP - CVP}{CI} \bullet 80$$

- Pulmonary vascular resistance index

$$PVRI = \frac{MPAP - PCWP}{CI} \bullet 80$$

- Left cardiac work index

$$LCWI = CI \bullet MAP \bullet 0.0144$$

- Right cardiac work index

$$RCWI = CI \bullet MPAP \bullet 0.0144$$

- Arterial oxygen content

$$C_aO_2 = (1.38 \bullet Hgb \bullet SatO_2) + (0.0031 \bullet P_aO_2)$$

- Oxygen delivery index

$$DO_2I = CI \bullet C_aO_2 \bullet 10$$

- Mixed venous oxygen content

$$C_{MV}O_2 = (1.38 \bullet Hgb \bullet Sat_{MV}O_2) + (0.0031 \bullet P_{MV}O_2)$$

- Oxygen consumption index

$$VO_2I = CI \bullet C(a-v)O_2 \bullet 10$$

- Oxygen extraction ratio

$$O_2ER = \frac{C_aO_2 - C_{MV}O_2}{C_aO_2} \bullet 100$$

FIGURE E1. Oxygen delivery index (DO_2I) before the operation (*pre-op*), before cardiopulmonary bypass (*CPB*), and within the first 24 hours postoperatively. Data are presented as means $\pm$ standard error of the mean. Mixed-effects modeling with protection type as fixed and time as random factor was used (protection, $P = .02$). * $P < .05$ between groups at the same time point ($n = 20$ for every group).

TABLE E1. Patient characteristics

	Diazoxide group (n = 20)	Placebo group (n = 20)	P value
Age (y)	61 $\pm$ 8	64 $\pm$ 7	.2
Sex			
Male	5 (25%)	8 (40%)	.5
Female	15 (75%)	12 (60%)	.5
CCS class	2.6 $\pm$ 0.7	2.4 $\pm$ 0.8	.6
Ejection fraction (%)	51 $\pm$ 5	50 $\pm$ 5	.5
Previous myocardial infarction	10 (50%)	11 (55%)	1.0
Coronary artery disease risk factors			
Hypertension	12 (60%)	14 (70%)	.7
Hyperlipidemia	12 (60%)	13 (65%)	1.0
Smokers			
Current	3 (15%)	4 (20%)	1.0
Past	12 (60%)	7 (35%)	.2
Diabetes mellitus	0	0	
Treatment			
ACEI	14 (70%)	17 (85%)	.5
β -Blockers	17 (85%)	18 (90%)	1.0
Ca ²⁺ blockers	3 (15%)	2 (10%)	1.0
Statins	11 (55%)	16 (80%)	.2
Aspirin	1 (5%)	0	1.0

CCS, Canadian Cardiovascular Society; ACEI, angiotensin-converting enzyme inhibitors.