Pyruvate-fortified cardioplegia suppresses oxidative stress and enhances phosphorylation potential of arrested myocardium

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Knott, E. Marty, Myoung-Gwi Ryou, Jie Sun, Abraham Heymann, Arti B. Sharma, Yu Lei, Mirza Baig, Robert T. Mallet, and Albert H. Olivencia-Yurvati. Pyruvate-fortified cardioplegia suppresses oxidative stress and enhances phosphorylation potential of arrested myocardium. Am J Physiol Heart Circ Physiol 289: H1123–H1130, 2005. —Cardioplegic arrest for bypass surgery imposes global ischemia on the myocardium, which generates oxysradicals and depletes myocardial high-energy phosphates. The glycolytic metabolite pyruvate, but not its reduced congener lactate, increases phosphorylation potential and detoxifies oxysradicals in ischemic and postischemic myocardium. This study tested the hypothesis that pyruvate mitigates oxidative stress and preserves the energy state in cardioplegically arrested myocardium. In situ swine hearts were arrested for 60 min with a 4:1 mixture of blood and crystalloid cardioplegia solution containing 188 mM glucose alone (control) or with additional 23.8 mM lactate or 23.8 mM pyruvate and then reperfused for 3 min with cardioplegica-free blood. Glutathione (GSH), glutathione disulfide (GSSG), and energy metabolites [phosphocreatine (PCr), creatine (Cr), Pi] were measured in myocardium, which was snap frozen at 45 min arrest and 3 min reperfusion to determine antioxidant GSH redox state (GSH/GSSH) and PCr phosphorylation potential ([PCr]/[Cr][Pi]). Coro-
23.8 mM lactate or 23.8 mM pyruvate. Oxidative stress was assessed by measuring 8-isoprostane concentration in coronary sinus blood. Left ventricular myocardial biopsies were obtained during arrest and at 3 min reperfusion for measurement of glutathione redox state and energy metabolites. These experiments demonstrated that pyruvate-fortified cardioplegia dampens oxidative stress and bolsters endogenous antioxidant defenses. Additionally, administration of pyruvate-fortified cardioplegia during arrest enhanced myocardial energetic recovery on reperfusion. These results extend the previously established benefits of pyruvate on the heart to a large animal model of cardioplegic arrest.

**METHODS**

Animal experimentation was approved by the Institutional Animal Care and Use Committee of the University of North Texas Health Science Center and was conducted in accordance with the Guide to the Care and Use of Laboratory Animals (National Institutes of Health 85-23, Revised 1996). Fifty-five adult domestic swine of either sex weighing 45–60 kg were randomly assigned to one of four experimental groups.

**Surgical procedures.** After an overnight fast, pigs were premedicated with ketamine (10 mg/kg im) and xylazine (1 mg/kg im). After sedation, anesthesia was induced with propofol (2 mg/kg iv) and maintained by mechanical ventilation with 1–3% isoflurane supplemented with O2 and propofol (1 mg·kg⁻¹·h⁻¹ iv). Cannulas were placed in the femoral arteries to monitor arterial pressure (model 1290C pressure transducer, Hewlett-Packard) and sample arterial blood. Plasma Lyte A (Baxter Healthcare, Deerfield, IL) was administered via a femoral vein to help maintain adequate blood volume. After median sternotomy, a pericardial cradle was fashioned for exposure and support of the heart. A catheter was placed through the inferior vena cavae to effectively isolate the heart. Initial induction of cardiac arrest was accomplished with antegrade infusion, via the aortic cannula, of a minimum of 400 ml cold (4°C) blood cardioplegia (4 blood:1 crystalloid solution, vol/vol). Arrest was maintained by infusing 100–150 ml cardioplegia into the aortic root at 20-min intervals. After the 60-min arrest, the heart was reperfused for 3 min at 100–140 ml/min with cardioplegia-free blood via the aortic cannula. Perfusion pressure (model 1290C pressure transducer, Hewlett-Packard) and flow rates (Transonic Flowprobe, Transonic Systems) were monitored during delivery of cardioplegia and cardioplegia-free blood. Perfusion pressure was maintained between 65 and 75 mmHg.

**Cardioplegia solutions.** All cardioplegia solutions were prepared aseptically the morning of the experiment. The control cardioplegia group contained 0.9% NaCl as the main diluent. The lactate cardioplegia was prepared using Ringer lactate solution. The pyruvate cardioplegia group was administered a pyruvate-fortified, Ringer-based cardioplegia solution, prepared by diluting sodium pyruvate powder (Sigma, St. Louis, MO) in sterile 0.9% NaCl. Pyruvate and lactate concentrations were 23.8 mM in the respective cardioplegia solutions. Electrolytes and other constituents were added to each solution to match the composition of the control cardioplegia. NaCl was added to control cardioplegia to equal the molar amounts of sodium lactate and sodium pyruvate added to the lactate and pyruvate cardioplegia solutions. All three crystalloid cardioplegia solutions contained 104 mM NaCl, 135 mM NaHCO₃, 91 mM KCl, 6 mM CaCl₂, 188 mM glucose, 68 µU/l insulin, and 676 mg/l lidocaine. Final pH was 7.6. Crystalloid solutions were combined with 4 vol blood before administration. Dilution of crystalloid in plasma lowered the pyruvate and lactate concentrations to 6–7 mM, well within the optimally cardioprotective pyruvate concentration range of 5–10 mM defined in isolated hearts (5).

**Arterial fuels, blood gases, and plasma 8-isoprostane.** Blood samples were collected before initiation of cardioplegic arrest, at initial arrest, at 20 and 40 min arrest, and at 1 and 3 min reperfusion. Arterial blood samples were obtained from the femoral artery before arrest and from the aortic perfusion line carrying blood cardioplegia during arrest or whole blood during reperfusion. Coronary venous blood was sampled from the coronary sinus. Blood gases, pH, and HCO₃⁻ were measured in an Instrumentation Laboratory model 1730 blood gas analyzer. Pyruvate concentrations in plasma extracts were measured by colorimetric assays (49) in a Shimadzu Instruments model UV-1601 spectrophotometer. Glucose and lactate concentrations were measured in a Yellow Springs Instruments model 2300 t-lactate analyzer. Glucose and lactate concentrations reported by this analyzer have been verified by spectrophotometry. 8-Isoprostane was measured in coronary sinus plasma using a competitive immunoassay kit (Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s instructions. Plasma 8-isoprostane concentrations were normalized for whole blood hemoglobin concentration and expressed as a percentage of prearrest baseline.

**Myocardial metabolites, antioxidant redox state, and cytosolic energy state.** Snap-frozen biopsies (~5 g) of the left ventricular apex were taken in situ by compressing tissue in Wollenberger tongs precooled in liquid N₂ (15). Biopsies were stored at −80°C until metabolite extraction. Three experimental series were performed. In the first series, myocardium was sampled at 45 min of arrest (8...
In the second series, myocardium was biopsied at 3 min reperfusion in 7 control, 10 lactate, and 8 pyruvate cardioplegia experiments. The third series was a sham control protocol (n = 6) in which myocardium was sampled 1 h and 45 min after sternotomy but without cardiac instrumentation or cardioplegic arrest. Experiments were terminated after biopsy.

Myocardial metabolites were extracted (27, 41, 42) and assayed spectrophotometrically (3). Phosphocreatine phosphorylation potential, i.e., 

\[
\frac{[\text{PCr}]}{([\text{Cr}][\text{Pi}])}
\]

provided a measure of myocardial energy state (2, 45). Intracellular P_i was determined as previously reported (25). GSH and GSSG in myocardial extracts were measured in a Shimadzu Instruments model LC-10AT high-performance liquid chromatography system equipped with a fluorescence detector (29). The GSH/GSSG was taken as a measure of the antioxidant redox potential of the myocardium.

**Statistical analysis.** Data are reported as means ± SE. Arterial blood gases, pH, and \(\text{HCO}_3^-\), as well as plasma metabolite and \(8\text{-isoprostane}\) data at different time points within each group, were compared by repeated measures ANOVA. Between-group comparisons of these variables at selected time points and myocardial metabolite values were accomplished by factorial ANOVA. When ANOVA detected statistical significance, post hoc multiple comparisons versus sham values were made using Dunn’s method, and other between-group comparisons were performed using Student-Newman-Keuls multiple comparison test. Student’s t-tests were performed for within-group comparisons of data collected at 3 min reperfusion versus 45 min arrest. \(P\) values <0.05 were considered statistically significant.

**RESULTS**

**Arterial acid-base chemistry and hematocrit.** Arterial blood gases, pH, and \(\text{HCO}_3^-\) are reported in Fig. 2. Infusion of \(\text{HCO}_3^-\) in crystalloid cardioplegia controlled arterial pH (Fig. 2A) and increased arterial P_{CO2} via the carbonic anhydrase equilibrium (Fig. 2B), in parallel with increased \(\text{HCO}_3^-\) concentration (Fig. 2C). Arterial O_2 saturation (Fig. 2D) was maintained at ~97% in all three cardioplegia groups. Hematocrit (Fig. 2E) fell during arrest due to fluid administration and to the addition of the crystalloid cardioplegia component; accordingly, O_2 content (Fig. 2F) also decreased during arrest and reperfusion.

**Arterial and tissue carbohydrates.** Hearts were arrested with one of three cardioplegia solutions, glucose alone (control) or glucose with additional 23.8 mM lactate or pyruvate. Inclusion of pyruvate in crystalloid cardioplegia produced a plasma pyruvate concentration of about 7 mM in blood cardioplegia.

**Fig. 2.** Arterial blood gases and acid-base chemistry. pH (A), P_{CO2} (B), HCO_3^- (C), O_2 saturation (D), hematocrit (E), and O_2 content (F) were measured at prearrest baseline (BL), at 20-min intervals during arrest, and at 1 and 3 min of reperfusion. Values are means ± SE. *\(P\) < 0.05 vs. baseline in all three groups; †\(P\) < 0.05 vs. baseline, control group only.
Plasma pyruvate concentration did not increase in the control or lactate cardioplegia groups during arrest. Similarly, plasma lactate concentration increased to 6 mM in the lactate cardioplegia group but was only slightly elevated in the control and pyruvate cardioplegia groups (Fig. 3B). Arterial glucose concentration during arrest averaged about 40 mM in each of the three cardioplegia groups (Fig. 3C).

Myocardial uptake and release of carbohydrate substrates are reported in Fig. 4. Baseline rates are not reported because total coronary flow was not measured before arrest. Myocardial pyruvate uptake (Fig. 4A) was between 0.4 and 0.5 mmol/min in the pyruvate group during cardioplegic arrest. There was no net pyruvate uptake in the pyruvate cardioplegia group during cardioplegia-free reperfusion nor in the control and lactate cardioplegia groups at any time. Lactate was released from the heart throughout the protocol in the control and pyruvate cardioplegia groups, but a brief period of net lactate uptake was seen during early arrest in the lactate group (Fig. 4B). Glucose (Fig. 4C) was taken up during arrest in all three groups. On reperfusion, net glucose release occurred as glucose-enriched cardioplegia was washed out of the organ by cardioplegia-free arterial blood.

Pyruvate (Fig. 5A), lactate (Fig. 5B), and citrate (Fig. 5C) contents were measured in arrested and reperfused myocardium and compared with respective contents in nonarrested sham hearts. Pyruvate content in the sham group was 0.32 ± 0.11 μmol/g dry tissue wt. Pyruvate content in the control and lactate groups did not change during arrest or reperfusion. During arrest, pyruvate content increased fivefold in the pyruvate cardioplegia group (1.50 ± 0.39 μmol/g dry tissue wt) but...
was no longer significantly elevated after reperfusion (0.80 ± 0.17 μmol/g dry tissue wt) due to pyruvate clearance by washout and metabolism. Myocardial lactate content doubled in the control cardioplegia group and increased fivefold in the lactate and pyruvate groups during arrest. Although lactate content did not change after reperfusion of the lactate and pyruvate groups, it increased threefold in the control group to a level similar to that in the other groups.

Pyruvate carboxylase (36, 37) and malic enzyme (40) carboxylate pyruvate to form the tricarboxylic acid cycle intermediates oxaloacetate and malate, respectively, culminating in the formation of citrate. Citrate content increased 2.5-fold in the control and lactate groups during arrest and remained elevated after reperfusion. Pyruvate cardioplegia produced an even greater increase in citrate content during arrest and reperfusion versus control and lactate cardioplegia.

Coronary sinus 8-isoprostane. To assess oxidative stress during cardioplegic arrest and reperfusion, the lipid peroxidation product 8-isoprostane (30, 43) was measured in coronary sinus plasma. 8-Isoprostane concentrations were normalized to hemoglobin concentration of coronary sinus blood to control for hemodilution and reported as percentages of prearrest baseline (Fig. 6). Pyruvate cardioplegia suppressed 8-isoprostane release in the arrested and reperfused heart, relative to control cardioplegia. This effect was most striking during reperfusion when 8-isoprostane increased sixfold in the control cardioplegia group. Unexpectedly, lactate cardioplegia also dampened oxidative stress compared with control but only during reperfusion.

GSH antioxidant system. GSH is the major intracellular antioxidant in the heart. The GSH/GSSG ratio is an index of the global redox state of the antioxidant defenses of myocardium (38). Accordingly, GSH, GSSG (Fig. 7A), and GSH/GSSG (Fig. 7B) were determined in arrested and reperfused myocardium and compared with respective values in nonarrested sham hearts. During cardioplegic arrest, GSH content was maintained in all three groups. Although not statistically significant, GSSG tended to fall in the control and pyruvate groups, thus raising the GSH redox state. Reintroduction of cardioplegia-free arterial blood to the myocardium on reperfusion induced oxidative stress: GSH content fell by ~25% and GSSG increased in all three cardioplegia groups. Thus GSH redox state was depressed after reperfusion.

Energy metabolites and phosphorylation potential. The impact of cardioplegic arrest and reperfusion on myocardial energy state was examined by measuring creatine kinase reactants and phosphorylation potential. ATP was maintained near the sham control value during arrest and reperfusion in all three groups (Table 1). Phosphocreatine (PCr) and creatine (Cr) contents did not differ appreciably among the cardioplegia groups.

PCr phosphorylation potential ([PCr]/[Cr][Pi]), an index of cytosolic energy state according to the creatine kinase

![Fig. 5. Myocardial pyruvate, lactate, and citrate contents. Pyruvate (A), lactate (B), and citrate (C) contents were measured in snap-frozen left ventricular biopsies taken at 45 min arrest and at 3 min reperfusion in control, lactate, and pyruvate cardioplegia groups, and at 105 min poststernotomy in nonarrested sham hearts. Values are means ± SE. *P < 0.05 vs. sham; †P < 0.05 vs. respective values in the same group at 45 min arrest; ‡P < 0.05 vs. sham, control, and lactate.](http://ajpheart.physiology.org/)

![Fig. 6. Coronary sinus 8-isoprostane concentration. 8-Isoprostane was measured in coronary sinus plasma. Measured concentrations were divided by hemoglobin concentration in coronary sinus blood to control for varying degrees of hemodilution and expressed as percentages of prearrest baseline. Actual baseline values were 3.77 ± 0.25, 2.70 ± 0.59, and 5.10 ± 1.75 ng 8-isoprostane/g hemoglobin in the control, lactate, and pyruvate groups, respectively. Values are means ± SE. *P < 0.05 vs. baseline; †P < 0.05 vs. control; ‡P < 0.05 vs. lactate.](http://ajpheart.physiology.org/)
equilibrium (45), permitted assessment of the effects of cardioplegic arrest and reperfusion on myocardial energy reserves. Phosphorylation potential in all three groups tended to increase by 45 min arrest (Fig. 8A) despite interruption of coronary flow. On reperfusion, phosphorylation potential returned to prearrest baseline in the control and lactate cardioplegia groups. Pyruvate cardioplegia maintained the higher phosphorylation potential even after reperfusion at a level (275 ± 38 M⁻¹) roughly double that of the control (148 ± 32 M⁻¹), lactate (116 ± 16 M⁻¹), and nonarrested sham (109 ± 8 M⁻¹) groups. This enhancement of phosphorylation potential was due in large part to decreased intracellular Pi concentration (Fig. 8B).

The wet-to-dry ratio (Table 1) monitors myocardial water content and tissue edema. The ratio did not change during arrest but increased in all three groups after 3 min reperfusion. Thus reperfusion of cardioplegically arrested myocardium appeared to produce some moderate edema, irrespective of the fuel composition of the cardioplegia.

DISCUSSION

A recent clinical trial (33) demonstrated marked, sustained enhancement of postsurgical cardiac function in patients administered pyruvate- versus lactate-fortified cardioplegia during CPB. This investigation aimed to delineate the mechanisms of pyruvate protection of the cardioplegically arrested myocardium. Pyruvate cardioplegia produced a plasma pyruvate concentration of ~7 mM, well within the optimally cardioprotective range of 5–10 mM pyruvate defined in isolated perfused heart preparations (5). Myocardial energy and antioxidant redox states were measured during cardioplegic arrest and at 3 min reperfusion, a time of intense oxidative stress.

Table I. Myocardial creatine kinase reactants

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<th>n</th>
<th>ATP</th>
<th>PCR</th>
<th>Cr</th>
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<td>90±8</td>
<td>5.55±0.18††</td>
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Values are means ± SE; n, number of pigs. ATP, phosphocreatine (PCr), and creatine (Cr) contents (µmol/g dry tissue wt) were measured in left ventricular tissue biopsied at 45 min arrest and 3 min reperfusion. Mass ratios of wet to dry tissue are also listed. *P < 0.05 vs. sham; †P < 0.05 vs. respective values in the same group at 45 min arrest; ‡P < 0.05 vs. lactate.

**Fig. 7.** Myocardial glutathione redox state. A: glutathione (GSH, solid bars) and glutathione disulfide (GSSG, hatched bars) contents were measured in left ventricular myocardium at 45 min arrest and 3 min reperfusion in the cardioplegia groups and at 105 min poststernotomy in nonarrested sham hearts. GSH and GSSG values are plotted on different scales. B: glutathione redox state (GSH/GSSG) at 45 min arrest (black bars) and 3 min reperfusion (hatched bars) was computed from GSH and GSSG contents. Values are means ± SE. *P < 0.05 vs. sham; †P < 0.05 vs. respective values in the same group at 45 min arrest; ‡P < 0.05 vs. lactate.

**Fig. 8.** Myocardial phosphorylation potential and Pi. A: phosphocreatine phosphorylation potential ([PCr]/[Cr][Pi]) was computed from phosphocreatine (PCr) and creatine (Cr) contents (see Table 1) and Pi concentration (B) measured in left ventricular myocardium sampled at 45 min arrest and 3 min reperfusion. Values are means ± SE. *P < 0.05 vs. sham; †P < 0.05 vs. respective values in the same group at 45 min arrest; ‡P < 0.05 vs. sham, control, and lactate.
Antioxidant effects of pyruvate. ROS are formed as a result of myocardial ischemia and reperfusion in animal models (34) and in humans (9) and have been implicated in postischemic myocardial dysfunction (9, 22, 46). In this study, pyruvate cardioplegia minimized myocardial 8-isoprostane release, a measure of lipid peroxidation, during arrest and reperfusion. Pyruvate also increased the GSH redox state of the heart during arrest. The concentration of 8-isoprostane in systemic arterial plasma of patients increases during CPB (43). 8-Isoprostane concentration also increased in coronary sinus blood 30 min after CPB in dogs (12). In addition, intravenous administration of the pharmacological antioxidant N-acetylcysteine throughout CPB reduced 8-isoprostane, preserved systolic function, and hastened myocardial edema resolution (12). Although cardiac performance could not be studied in this investigation, the established correlation between reduced oxidative stress and improved function could help explain the marked enhancement of myocardial function in patients arrested with pyruvate-fortified cardioplegia (33).

Pyruvate cardioplegia, more so than control and lactate cardioplegia, increased myocardial citrate content. Anaerobic pyruvate carboxylation generates the tricarboxylic acid cycle intermediates malate and oxaloacetate; condensation of oxaloacetate with acetyl CoA yields citrate (26). Citrate remained elevated in the pyruvate cardioplegia group at 3 min reperfusion even though pyruvate content had decreased by this time. Citrate generates NADPH, the source of reducing power to maintain GSH, through two pathways. First, citrate is converted to isocitrate, generating substrate for NADP⁺-dependent isocitrate dehydrogenase (1). Second, as citrate accumulates, it constrains phosphofructokinase activity (14), causing glucose-6-phosphate to accumulate (41) and thereby providing substrate for the NADPH-generating hexose monophosphate shunt.

Lactate does not possess any known antioxidant properties (2), so the mechanism of lactate suppression of postarrest lipid peroxidation is unclear. Lactate could be rapidly converted to its antioxidant congener pyruvate by lactate dehydrogenase, through its conversion to lactate, pyruvate oxidizes NADH to NAD⁺, which could relieve glycolytic constraint. In contrast, lactate accumulation could become limited by NADH accumulation and concomitant decrease in NAD⁺ (18). Pyruvate cardioplegia increased myocardial pyruvate and lactate contents fivefold during arrest. Through its conversion to lactate, pyruvate oxidizes NADH to NAD⁺, which could relieve glycolytic constraint. In contrast, lactate oxidation generates NADH, which could further impair glycolysis during arrest.

Limitations. This novel in situ heart preparation permits direct examination of the effects of cardioplegia on the organ. However, effectively isolating the heart from the organism by cross-clamping the great vessels precludes study of the systemic response to the stresses of CPB. In addition, only 1.2 liters of arterial blood could be safely withdrawn from the pig without provoking circulatory collapse despite replacement with equal volume of isotonic saline. The volume of blood available after blood cardioplegia administration was only sufficient for 3 min of reperfusion, therefore, postarrest recovery of cardiac performance could not be studied. Blood from donor animals was not administered to avoid the proinflammatory and pro-oxidant effects of allogeneic blood.

In conclusion, compared with control and lactate-fortified cardioplegia solutions, pyruvate cardioplegia significantly reduced oxidative stress in the heart during cardioplegic arrest. Moreover, antecedent administration of pyruvate or lactate cardioplegia during arrest suppressed the reperfusion burst of lipid peroxidation. A metabolic antioxidant, pyruvate enhanced GSH/GSSG antioxidant redox state during arrest. GSH/GSSG fell on reperfusion in all three cardioplegia groups. Phosphorylation potential was maintained or even enhanced during arrest in all three groups but fell after reperfusion in the control and lactate cardioplegia groups. Antecedent pyruvate cardioplegia bolstered myocardial phosphorylation potential even after reperfusion.

This investigation demonstrated for the first time that pyruvate-fortified cardioplegia reduces oxidative stress and preserves myocardial energy state in a large animal model of cardioplegic arrest. Further study of the beneficial effects of pyruvate on the heart during cardiopulmonary bypass is warranted.

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GRANTS

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