Brief Increase in Carbohydrate Oxidation After Reperfusion Reverses Myocardial Stunning in Conscious Pigs

Raymond K. Kudej, DVM, PhD; Lawrence T. White, BS; Amelia B. Kudej, BS; Stephen F. Vatner, MD; E. Douglas Lewandowski, PhD

Background—Previous studies have examined only acute effects of enhanced glucose oxidation on postischemic myocardium. The goal of the present study was to examine prolonged functional recovery subsequent to postischemic, intracoronary pyruvate dehydrogenase kinase inhibition with dichloroacetate (DCA) of stunned myocardium in conscious pigs.

Methods and Results—Myocardial stunning was induced in conscious pigs by coronary stenosis, ie, 40% reduction of coronary blood flow for 90 minutes, followed by full reperfusion. After the initial peak, but during early reactive hyperemia (5 minutes of reperfusion), 1 hour of intracoronary infusion at 20% of measured coronary blood flow was begun using 20 mmol/L [2-13C]glucose without (n=4) or with (n=5) 20 mmol/L DCA. Coronary stenosis resulted in similar reduction in wall thickening in both untreated (−53±3% from 3.27±0.22 mm, n=9) and DCA (−51±3% from 3.08±0.15 mm, n=5) groups. During reperfusion, DCA increased glucose oxidation 10-fold. In the absence of DCA, myocardial stunning was observed; ie, wall thickening was reduced by 48±3% at 1 hour of reperfusion and did not fully recover for 48 hours. In contrast, in DCA pigs, myocardial stunning was ameliorated (P<0.05).

Conclusions—Transient metabolic intervention within a clinically relevant time after ischemia eliminates myocardial stunning in conscious pigs during augmented carbohydrate oxidation and provides sustained benefits in contractile recovery. (Circulation. 2002;106:2836-2841.)

Key Words: stunning, myocardial I reperfusion I ischemia I glucose

Enhanced fatty acid oxidation after ischemia and reperfusion is associated with decreased myocardial function and cardiac efficiency.1 Importantly, a shift from fatty acid to carbohydrate oxidation has been shown to be beneficial to myocardial recovery after ischemia, and numerous pharmacological interventions have been used to promote glucose oxidation in this setting, including glucose-insulin-potassium,2-4 inhibition of mitochondrial β-oxidation,4 inhibition of carnitine palmitoyltransferase I,5,6 and pyruvate dehydrogenase (PDH) complex activation with L-carnitine7 or dichloroacetate (DCA).8-12 Additional regulatory sites suggested to potentially enhance glucose oxidation and function in posts ischemic myocardium include enhanced glucose transporter translocation to the sarcolemma, activation of acetyl-CoA carboxylase,13 and inhibition of malonyl-CoA decarboxylase.14 DCA promotes pyruvate oxidation by inhibiting PDH kinase and thereby maintaining PDH in the active dephosphorylated state.15,16 The increase in carbohydrate oxidation then also inhibits fatty acid oxidation via increased levels of malonyl-coenzyme A.17,18 Recently, the mechanism by which increased glucose oxidation promotes enhanced posts ischemic recovery has been linked to the recovery of intracellular pH and cytosolic redox state of the myocardium.11,19 However, whether posts ischemic functional improvement after PDH stimulation using DCA is sustained beyond the initial treatment period has not been determined in previous investigations because of the limitations of the experimental models. Functional recovery during brief, ie, short-term, reperfusion periods using buffer-perfused isolated heart preparations has been assessed most frequently, either after no-flow8,9,15,20,21 or low-flow22 ischemia. Additionally, in the few studies that have used in vivo preparations, DCA has been administered systemically in acute, open-chest models.23-26 Interestingly, intracoronary administration of DCA during ischemia induced by moderate reduction of coronary blood flow in anesthetized swine did not improve systolic function; however, postischemic recovery was not assessed in this study.27

The goal of the present study was to examine effects of postischemic, intracoronary pyruvate dehydrogenase (PDH) kinase inhibition on functional recovery of stunned myocar-
dium in conscious pigs. By using conscious, chronically instrumented pigs, this study for the first time could examine prolonged recovery subsequent to intervention. Additionally, the present study demonstrates the potential benefits of enhanced glucose oxidation in a clinically relevant period during reperfusion after myocardial ischemia.

Methods

Twenty-three domestic swine weighing 28.2 ± 0.9 kg were sedated with telazol 5 mg/kg (IM) and atropine 0.05 mg/kg (IM). General anesthesia was maintained with isoflurane (0.5 to 1.5 vol %) after tracheal intubation. The animals were trained and instrumented using sterile surgical technique, and hemodynamic recordings were made as previously described.28 Using sterile surgical technique, a left thoracotomy was performed at the 5th intercostal space. Tygon catheters (Norton Plastics) were implanted in the descending aorta and in the left atrium for measurement of pressures and radioactive microsphere injection. A solid-state miniaturized pressure gauge was implanted in the left ventricular (LV) cavity to obtain LV pressure and dP/dt. The left anterior descending (LAD) coronary artery was isolated, and a flow transducer (Transonics Systems Inc) and hydraulic occluder, made of Tygon tubing, were implanted. Additionally, 2 pairs of ultrasonic crystals were implanted transmurally across the LV free wall, in the anterior and in the posterior regions, for measurement of regional wall thickness. The left main coronary artery was dissected carefully proximal to its bifurcation, and a coronary artery catheter was placed into the LAD coronary artery as previously described.29 A Silastic catheter was placed retrograde into the coronary vein as close to the left anterior descending coronary artery as anatomically possible for myocardial venous sampling. Animals used in this study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, revised 1996). Vallium was administered at 0.5 to 1.0 mg/kg for tranquillization before initiation of the experimental protocol and additionally as required, ie, if the pigs became agitated transiently.

Experimental Protocols

The experiments in conscious pigs were initiated after a 1-week recovery period after surgical instrumentation. After global and regional baseline hemodynamic data were recorded, coronary stenosis (CS) was induced by injecting the hydraulic occluder to reduce coronary blood flow (CBF) by ~40%. The degree of coronary blood flow reduction was then continuously monitored and sustained for 90 minutes.

Glucose, enriched with carbon-13 ([1-13C]), was infused into the LAD without and with DCA to determine whether DCA was effective at augmenting myocardial glucose oxidation in the closed-chest, conscious pig. A short-term recovery protocol on the in situ pig heart involved 1.5-hour CS with 5 minutes of reperfusion followed by 1 hour of intracoronary infusion of either [2-13C]-labeled glucose (20 mmol/L) alone (n = 4) or [2-13C]-labeled glucose (20 mmol/L) and DCA (20 mmol/L) (n = 5). The rate of infusion for each was at 20% of measured LAD coronary blood flow, for a final dilution of 20% (5-min infusion period). Minimal coronary blood flow (CBF) was maintained to ensure 20% of measured LAD coronary blood flow, for a final dilution of 20% (5-min infusion period). Minimal coronary blood flow (CBF) was maintained to ensure that the [2-13C]glucose concentration to 4 mmol/L in the blood that was perfusing the LAD bed. In addition, a long-term recovery protocol involved 1.5 hours of CS with 5 minutes of reperfusion followed by either reperfusion without intracoronary infusion (n = 8) or 1 hour of intracoronary infusion of saline (n = 1), which together served as untreated controls (n = 9), or 1.5 hours of CS with 5 minutes of reperfusion followed by 1 hour of intracoronary infusion of DCA (20 mmol/L, 20% LAD flow, n = 5). The saline, sham infusion did not influence stunning. These animals were then monitored for 4 days after CS.

At the end of each short-term protocol during substrate perfusion, the pigs were killed with bolus infusion of sodium pentobarbital (100 mg/kg) into the left atrial catheter. A thoracotomy was performed immediately, and a transmural section of the anterior LV wall was excised, divided rapidly into endocardial and epicardial segments, and frozen in a large aluminum tissue clamp precooled in liquid nitrogen. Killing and tissue sampling were performed routinely in <1 minute. Frozen tissue samples were then transferred to vials in liquid nitrogen and stored at −80°C before NMR determination of labeled glucose oxidation.

Additional samples were taken from the ischemic and nonischemic areas for regional myocardial blood flow determination using the radioactive microsphere technique as previously described.28 Radioactive microspheres were administered at baseline and twice during CS (30 and 90 minutes) to confirm blood flow reduction during CS. The samples were counted in a gamma counter (Searle Analytical) with appropriately selected energy windows. After correction of counts for background and crossover, regional myocardial blood flow was obtained and expressed as mL/min per g of tissue. After a 4-day recovery period, the long-term recovery animals were killed with an acute overdose of pentobarbital, and the heart was removed and samples taken for tissue blood flow analysis.

In Vitro NMR Spectroscopy and Tissue Chemistry

Tissue metabolites were extracted from 1 g of frozen myocardium from the LAD bed of the left ventricle using 7% perchloric acid. Total tissue glutamate was assayed and the fraction of labeled glutamate was determined as previously described.30 Acid extracts of 1 g samples of myocardium were freeze dried and reconstituted in 0.5 mL of water for in vitro NMR analysis. In vitro 13C NMR spectra were acquired in a 5-mm probe with proton decoupling during summation of 4000 scans (45-degree pulse with 1.8-second interpulse delay) on a NMR spectrometer interfaced to a 9.4-Tesla, vertical bore, superconducting magnet (Bruker Instruments, Billerica, Mass). The presence of label at the 5-carbon resonance of glutamate indicated oxidation of the [2-13C]glucose, as previously described.31 The signal intensity from the enriched 5-carbon of glutamate was used to confirm the action of DCA on glucose oxidation.

Statistical Analysis

Comparison of results between groups was performed with the Student’s unpaired, two-tailed t test for comparison between 2 groups and an ANOVA test of variance for 3 or more groups. Differences between mean values were considered statistically significant at P < 0.05. Results are presented as mean ± SEM.

Results

Effects of Intracoronary DCA

Coronary Stenosis

Changes in CBF and ischemic zone wall thickening (WT) during 1.5-hour CS and 1-hour reperfusion in animals given either intracoronary [2-13C]glucose alone or DCA at 5 minutes after reperfusion are compared in Figure 1, whereas longer term recovery is represented in Figure 2. Hemodynamic and regional function measurements at baseline, during CS, and during recovery at 1 hour of reperfusion in the absence and presence of intracoronary DCA infusion are listed in Table 1. CBF was reduced by an average of 40 ± 1% during CS from a baseline of 27 ± 2 mL/min. Ischemic zone WT decreased by an average of 48 ± 3% from 3.3 ± 0.4 mm at 30 minutes of CS. Ischemic zone WT continued to decrease, ie, was reduced by 58 ± 4% from baseline, at the end of the 1.5-hour CS. Reductions in CBF and ischemic zone WT were similar during CS in animals that received DCA after reperfusion. There were moderate but significant changes in LV end-diastolic pressure and LV dP/dt during CS, noted in Table 1, whereas other hemodynamic variables did not change significantly with CS.
Reperfusion

Either DCA and [2-13C]glucose or [2-13C]glucose alone was administered beginning 5 minutes after reperfusion and maintained for 1 hour. Just before infusion, CBF was increased by 133 ± 16% in the group receiving DCA and was elevated similarly in the other group. WT was also reduced similarly in both groups (46 ± 4% versus 44 ± 2%) at 5 minutes after reperfusion. CBF remained elevated at 1 hour of reperfusion similarly in both groups. In the [2-13C]glucose alone group, myocardial stunning, as reflected by depressed ischemic zone

WT, did not recover during the 1-hour reperfusion period. However, in the animals receiving DCA, myocardial stunning was ameliorated (Figure 1).

Effects of 1-Hour DCA Infusion on Long-Term Recovery

Changes in ischemic zone WT during 1.5 hours of CS and 4 days of reperfusion are represented in Figure 2. Hemodynamic and regional function measurements averaged at baseline during CS and recovery at 12 hours and 4 days after coronary artery reperfusion are listed in Table 2. The changes during CS and during the first hour of reperfusion were similar to values observed in the corresponding short-term groups (Figure 2 and Table 2). In the pigs that did not receive DCA, WT remained depressed for 3 hours at a level similar to values at 1 hour of reperfusion and gradually recovered over the following 3 days, reflecting prolonged myocardial stunning. In contrast, the pigs receiving DCA did not exhibit prolonged, severe myocardial stunning. There was only transient myocardial stunning after cessation of the infusion, which rapidly recovered (Figure 2).

TABLE 1. Hemodynamics and Regional Myocardial Function at Baseline, During CS, and During Reperfusion (R) Without (n=4) and With DCA (n=5)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Ave CS</th>
<th>1-Hour R</th>
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<tbody>
<tr>
<td>CBF, mL/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- DCA</td>
<td>26.5 ± 5.8</td>
<td>16.0 ± 3.6*</td>
<td>60.8 ± 14.7*</td>
</tr>
<tr>
<td>+ DCA</td>
<td>32.5 ± 2.3</td>
<td>17.8 ± 1.8*</td>
<td>62.4 ± 12.5*</td>
</tr>
<tr>
<td>LV systolic pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- DCA</td>
<td>121 ± 4</td>
<td>118 ± 3</td>
<td>125 ± 7</td>
</tr>
<tr>
<td>+ DCA</td>
<td>115 ± 4</td>
<td>111 ± 3</td>
<td>114 ± 2</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- DCA</td>
<td>9.8 ± 0.3</td>
<td>15.3 ± 0.3*</td>
<td>12.3 ± 0.6</td>
</tr>
<tr>
<td>+ DCA</td>
<td>8.0 ± 0.6</td>
<td>14.3 ± 1.0*</td>
<td>10.8 ± 0.2</td>
</tr>
<tr>
<td>LV dP/dt, mm Hg/s</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- DCA</td>
<td>2905 ± 121</td>
<td>2527 ± 79*</td>
<td>2935 ± 115</td>
</tr>
<tr>
<td>+ DCA</td>
<td>2800 ± 148</td>
<td>2373 ± 134*</td>
<td>2760 ± 197</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- DCA</td>
<td>102 ± 5</td>
<td>102 ± 3</td>
<td>109 ± 5</td>
</tr>
<tr>
<td>+ DCA</td>
<td>99 ± 3</td>
<td>96 ± 3</td>
<td>98 ± 2</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- DCA</td>
<td>120 ± 5</td>
<td>122 ± 5</td>
<td>124 ± 5</td>
</tr>
<tr>
<td>+ DCA</td>
<td>121 ± 5</td>
<td>122 ± 7</td>
<td>122 ± 6</td>
</tr>
<tr>
<td>Ischemic wall thickening, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- DCA</td>
<td>3.33 ± 0.38</td>
<td>1.55 ± 0.17*</td>
<td>2.09 ± 0.31*</td>
</tr>
<tr>
<td>+ DCA</td>
<td>3.28 ± 0.30</td>
<td>1.57 ± 0.23*</td>
<td>3.09 ± 0.40†</td>
</tr>
<tr>
<td>Ischemic wall thickening, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- DCA</td>
<td>35.5 ± 3.5</td>
<td>17.8 ± 2.0*</td>
<td>22.3 ± 3.5*</td>
</tr>
<tr>
<td>+ DCA</td>
<td>32.5 ± 2.3</td>
<td>17.8 ± 1.8*</td>
<td>30.9 ± 3.1†</td>
</tr>
<tr>
<td>End Diastolic wall thickness, mm</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>- DCA</td>
<td>9.41 ± 0.55</td>
<td>8.75 ± 0.60</td>
<td>9.47 ± 0.59</td>
</tr>
<tr>
<td>+ DCA</td>
<td>10.13 ± 0.78</td>
<td>8.77 ± 0.94</td>
<td>9.98 ± 0.84</td>
</tr>
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</table>

*P < 0.05, different from baseline.
†P < 0.05, + DCA different from − DCA.
Myocardial Blood Flow Distribution Within the Area at Risk

Absolute values for tissue blood flows (mL/min per g) in the ischemic and nonischemic zones, for short-term recovery control (n=4) and DCA (n=4) groups as well as long-term control (n=7) and DCA (n=4) groups, are listed in Table 3. During CS, transmural tissue blood flow decreased similarly to that observed with flowmeter measurement. However, subepicardial blood flow did not decrease significantly during CS in any short- or long-term recovery group, whereas subendocardial blood flow decreased by 34% to 37% in each group.

Increased Glucose Oxidation

Glucose oxidation was increased in the reperfused myocardium by the infusion of DCA. Oxidation of [2-13C]glucose resulted in 13C enrichment of the tissue glutamate pool with detection of a corresponding 13C NMR signal from the 5-carbon position of glutamate at 182 ppm (Figure 3). Compared with the untreated hearts, administration of DCA increased the amount of glutamate produced from the oxidation of labeled glucose in both layers of myocardium. From the fractional 13C enrichment of the glutamate 5-carbon, the percentage of glutamate produced from oxidation of [2-13C]glucose in each group was 3.66±2.7% versus 37.0±6.7% in the epicardium and 2.0±1.0% versus 31.6±4.9% in the endocardium (P<0.01). These fractional enrichments occurred at similar glutamate content in both experimental groups ranging between 12 and 14 μmol/g dry weight.

Discussion

The goal of the present investigation was to determine whether brief metabolic regulation during the immediate postischemic period results in sustained improvement in functional recovery of stunned myocardium. The importance of the findings of the present study is related not only to the application of metabolic intervention in a clinical setting but may also provide some insight into the mechanism of myocardial stunning after a relatively brief period of moderate coronary blood flow reduction.

Although enhanced glucose oxidation using DCA has been shown to improve functional recovery with some models of ischemia and reperfusion, limitations of these models have precluded the assessment of the extent of these beneficial effects. Buffer-perfused isolated heart preparations have been used most frequently, either after no-flow8,9,15,20,21 or low-flow22 ischemia.
In the few studies that have used in vivo preparations, DCA has been administered systemically in acute, open-chest models in which, similar to the isolated hearts, it becomes difficult to interpret long-term functional response. Furthermore, systemic administration of DCA has been shown to decrease significantly arterial blood lactate level, which could affect lactate availability and functional response. The availability and use of lactate as a metabolic substrate has been previously shown to have an effect on the cellular redox state and functional response of postischemic myocardium to enhanced PDH activity. Interestingly, in a study by Mazer et al., intracoronary administration of DCA during ischemia induced by moderate reduction of coronary blood flow in anesthetized swine did not improve systolic function. Postischemic functional recovery was not assessed in this study; however, the study by Mazer et al. supports the importance of lactate availability and cellular redox state relative to enhanced functional recovery associated with DCA administration.

Importantly, this is the very first study to demonstrate that the beneficial effects of DCA and enhanced PDH activity on postischemic myocardial function are sustained well beyond the period of DCA administration. WT dropped transiently at 2 to 3 hours of reperfusion after delivery of DCA was stopped (Figure 2) but remained much improved over the untreated group during this period and then rebounded well above that of the untreated group. The surprising results that contractile function was improved several hours after infusion of DCA and that myocardial stunning was ameliorated early on and abolished by twelve hours reperfusion hold important implications for metabolic interventions to myocardial ischemia. Earlier work relied on protocols that administered DCA either immediately on initial reperfusion or during ischemia, when the myocardium is already oxygen deprived and unlikely to respond to any activation of oxidative enzymes. The use of a delayed infusion of DCA, to avoid high infusion rates to match initial hyperemia, also demonstrates that there is a window of time that exists for such metabolic intervention to be effective on reperfusion. How far out this treatment can be delivered remains to be determined. Additional significance is indicated in using a conscious, chronically instrumented pig model with intracoronary DCA administration. The present study provides a more clinically relevant model and avoids variables such as anesthesia and recent surgery, which may complicate interpretation of the results.

Because evidence of enhanced glucose oxidation appeared in the present study, these data demonstrate that PDH kinase inhibition with DCA was promoting glucose as an oxidative fuel in the postischemic, reperfused myocardium. Potential mechanisms by which enhanced glucose oxidation may attenuate the functional deficit associated with postischemic myocardium include reduction of H⁺ production from glycolysis uncoupled from glucose oxidation and maintenance of cellular ion homeostasis or by regulation of cellular redox state. Arterial or coronary venous lactate levels were not elevated at any time during reperfusion (arterial, 0.58±0.05

![DCA-treated vs Untreated](image-url)
from 1.28±0.13 mmol/L; venous, 0.47±0.06 from 0.62±0.10 mmol/L, n=6) with the model used in the present study. This finding is important because of the lack of beneficial effects of enhanced glucose oxidation with lactate as the source of pyruvate19 and because previous studies have documented high levels of regional and circulating lactate during ischemia and reperfusion in vivo.33,34

The significance of the present work is to validate the potential application of strategies to manipulate metabolism and promote functional recovery in the immediate postischemic period.4 Significantly, the beneficial effects of enhanced glucose oxidation, initiated in a clinically relevant time during early reperfusion, are maintained beyond the intracoronary administration of DCA using a conscious, in vivo model of myocardial stunning induced by moderate reduction in coronary blood flow. It remains to be determined whether these strategies are useful in all models of ischemia and reperfusion. Clearly, previous studies11,19,27 indicate that enhancement of glucose oxidation is not beneficial when lactate is readily available as a source for pyruvate. Furthermore, whether PDH stimulation is detrimental or beneficial when lactate is readily available as a source for pyruvate has been proposed in rodents.36 In view of the fact that the significance of the present work is to validate the potential application of strategies to manipulate metabolism and promote functional recovery in the immediate postischemic period.4

Acknowledgments

This study was supported in part by National Institutes of Health Grants HL49244, HL51678, HL62702, HL59139, HL33107, HL33065, HL69020, HL37404, and RR16592.

References


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Circulation. 2002;106:2836-2841
doi: 10.1161/01.CIR.0000039326.87475.98
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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