Evidence For a Role of Iron-Catalyzed Oxidants in Functional and Metabolic Stunning in the Canine Heart

Neil E. Farber, Gregory M. Vercellotti, Harry S. Jacob, Galen M. Pieper, and Garrett J. Gross

Brief (15-minute) coronary occlusion and subsequent reperfusion lead to prolonged functional and metabolic abnormalities (stunned myocardium). Previous work suggests that one factor responsible for this phenomenon is oxygen-derived free radicals. The formation of the highly reactive hydroxyl radical requires the presence of metal ions, most importantly iron. In the present study, the effect of the iron-chelator deferoxamine on the recovery of segment shortening (%SS) in the stunned myocardium was compared with a control group in barbital anesthetized dogs. Deferoxamine (500 mg intra-atrially) was administered 15 minutes prior to and throughout 15 minutes of coronary occlusion. %SS, regional myocardial blood flow, hemodynamics, and myocardial high-energy phosphates were measured. Areas at risk, collateral blood flow, and all hemodynamic parameters were similar between control and deferoxamine-treated animals. While deferoxamine did not prevent the loss of systolic wall function that occurred during ischemia, deferoxamine significantly improved the recovery of %SS at all times throughout reperfusion (3-hour %SS of pretreatment: control, 12 ± 11; deferoxamine, 65 ± 12), normalized endocardial ATP (percent of nonischemic area: control, 79 ± 5%, deferoxamine, 93 ± 6%), attenuated the reperfusion-induced rebound increase in phosphocreatine and prevented the increase in tissue edema at 3 hours after reperfusion. Thus, deferoxamine exhibited a cardioprotective action both metabolically and functionally in the stunned myocardium presumably by decreasing the redox cycling, and hence, the availability of catalytic iron for use in hydroxyl radical formation and for the initiation of lipid peroxidation. These data suggest a possible role for the hydroxyl radical as a mediator of postischemic abnormalities in reversibly injured tissue. (Circulation Research 1988;63:351–360)
tions, the major danger of their accumulation may be the production of hydroxyl radicals (·OH) via the iron-catalyzed Haber-Weiss or Fenton reactions\textsuperscript{13,15} as shown below.

$$\text{Fe}^{3+} + \cdot \text{O}_2 \rightarrow \text{Fe}^{2+} + \text{O}_2$$ \hspace{1cm} (1)

$$\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \cdot \text{OH}$$ \hspace{1cm} (2)

where ferric iron (Fe\textsuperscript{3+}) undergoes redox cycling and is reduced to ferrous iron (Fe\textsuperscript{2+}) upon reaction with \cdot \text{O}_2, followed by the reoxidation of Fe\textsuperscript{2+} by H\textsubscript{2}O\textsubscript{2} to generate the hydroxyl radical.

Recently, Bolli and coworkers\textsuperscript{5} showed an enhancement of posts ischemic functional recovery in the stunned canine myocardium with the use of the hydroxyl radical scavenger dimethylthiourea. Formation of hydroxyl radicals is dependent on the redox cycling of a metal catalyst that, in vivo, is most likely iron.\textsuperscript{13,15} Once formed, hydroxyl radicals are extremely reactive with virtually every type of biological molecule in their immediate vicinity. The purpose of the present study was to determine the importance of ·OH as a mediator of both functional and metabolic stunning and to investigate the role of iron in the prolonged abnormalities observed. Deferoxamine was used since, with relative specificity, it chelates the Fe\textsuperscript{3+} essential for the production of ·OH.\textsuperscript{16,17} The effects of deferoxamine on the loss of systolic wall function during 15 minutes of coronary occlusion and on the recovery of regional myocardial function and adenine nucleotide concentrations following 3 hours of reperfusion were investigated. Also, possible mechanisms by which this agent may be cardioprotective were analyzed, including improved hemodynamic state, increased coronary collateral blood flow, improved transmural myocardial flow distribution, preservation of high-energy phosphates, and a reduction in reperfusion-induced tissue edema.

Materials and Methods

General Preparation

Adult mongrel dogs (19–28 kg) of either sex were anesthetized with sodium barbital (300 mg/kg i.v.) and sodium pentobarbital (15 mg/kg i.v.), were ventilated at 10–15 breaths/min with a tidal volume of 15 ml/kg (Harvard Apparatus Respirator, South Natick, Massachusetts) and supplemented with 100% O\textsubscript{2}. An end-expiratory pressure was maintained with a trap to prevent atelectasis. Ventilation was adjusted to maintain arterial blood gases (model ABL2, Radiometer America, Westlake, Ohio) near normal physiological levels (control group: pH 7.42 ± 0.01; P\textsubscript{CO\textsubscript{2}}, 31 ± 1 mm Hg; P\textsubscript{O\textsubscript{2}}, 125 ± 12 mm Hg; deferoxamine group: pH 7.42 ± 0.02; P\textsubscript{CO\textsubscript{2}}, 32 ± 2; P\textsubscript{O\textsubscript{2}}, 127 ± 20). Body temperature was maintained at 38° C with a heating pad. A double-tipped pressure transducer catheter (model PC 771, Millar, Houston, Texas) was introduced into the aorta and left ventricle through the carotid artery to monitor aortic and left ventricular systolic and diastolic pressures. The first derivative of the left ventricular pressure pulse (dP/dt) was determined by electronic differentiation. The right femoral vein was cannulated for administration of subsequent anesthesia as needed. A left thoracotomy was performed at the fifth intercostal space, the lungs were retracted, the pericardium was incised, and the heart was suspended in a pericardial cradle. Approximately a 1.0-cm segment of the left anterior descending coronary artery (LAD) was dissected free from surrounding tissue distal to the first diagonal branch and a calibrated electromagnetic flow probe (model SP 7515, Statham, Hato Rey, Puerto Rico) was placed around the vessel. LAD coronary blood flow was measured with a flowmeter (Statham model 2202). A micrometer-driven mechanical occluder\textsuperscript{18} was placed distal to the flow probe so that there were no branches between the occluder and flow probe. The occluder was used to zero the flow probe and later occlude the artery. Limb lead II from the electrocardiogram and a tachograph (model 7P4F, Grass, Quincy, Massachusetts) were used for monitoring heart rate. All hemodynamics and regional segment function were recorded on a Grass Model 7 polygraph.

Myocardial Segment Shortening

Subendocardial segment function (percent segment shortening, %SS) was measured in the regions perfused by the LAD and the left circumflex coronary arteries using two pairs of ultrasonic crystals inserted into the subendocardium (approximately 10–15 mm apart and 7–9 mm deep). Crystal depths were verified at the end of each experiment. The crystal leads were connected to an ultrasonic amplifier that transformed the crystal-transmitted sound pulse into an electrical signal proportional to the distance between them while the tracings were monitored with an oscilloscope (Soltect Model 520). Changes in transmission time indicated the distance between the two crystals. Diastolic segment length (DL) was determined immediately prior to the rapid rise of positive dP/dt (onset of isovolumic contraction) and systolic segment length (SL) determined at peak negative dP/dt. %SS was calculated by use of the equation %SS = (DL – SL)/DL × 100. Segment length data were normalized by using a value of 10.0 for the control DL.\textsuperscript{19}

Regional Myocardial Blood Flow

Transmural myocardial tissue blood flow was determined by the radioactive microsphere technique as described previously.\textsuperscript{12} The left atrial appendage was cannulated for the administration of radioactive microspheres and the right femoral artery was cannulated for the withdrawal of a reference blood flow sample. Carbonized plastic microspheres (15 ±3 μm diameter) labeled with \textsuperscript{113}Ce, \textsuperscript{51}Cr, \textsuperscript{109}Ru, or \textsuperscript{99}Nb and suspended in isotonic saline with 0.01% Tween 80 were ultrasonicated and vortexed for 5 minutes each. Approximately 2–4 × 10\textsuperscript{6}
Metabolism Biopsy

obtained at the dye sites by the use of a cylindrical epicardium (identified by the methylene blue dye), liquid nitrogen. The frozen biopsies were divided described. The sample was clamped immediately cutting tool mounted onto a hand drill as previously mentioned into subepicardium, midmyocardium, and centrally ischemic regions (five pieces). The tissue samples were then weighed and all samples counted in a gamma counter (model 1195, Searle Analytic, Elk Grove, Illinois) to determine the activity of each isotope in each tissue and reference blood flow sample. Regional myocardial blood flow was calculated using a preprogrammed computer (Apple IIe) to obtain the true activity of each isotope in individual samples, and tissue blood flow was determined using the equation: 

\[ Q_m = Q_t \cdot C_{m} / C_{r} \]

where \( Q_m \) is myocardial blood flow (ml/min/g tissue), \( Q_t \) is rate of withdrawal of the reference blood flow sample (6.8 ml/min), \( C_r \) is activity of the reference blood flow sample (cpm), and \( C_m \) is activity of the tissue sample (cpm/g). Transmural tissue blood flow was calculated as the weighted average of the three layers in each region. Myocardial blood flow distribution (endoepicardialsubendocardial blood flow ratio, endo/epi) was determined by dividing the average subendocardial flow of each region by the average subepicardial flow. Extent of the area at risk was calculated by weighing the entire left ventricle and weighing all tissue pieces within the LAD perfused region.

Metabolism Biopsy

At 3 hours following reperfusion, immediately prior to sacrifice, a small area (1.5 cm) in normal and postischemic regions was painted with methylene blue dye. Transmural tissue samples were obtained at the dye sites by the use of a cylindrical cutting tool mounted onto a hand drill as previously described. The sample was clamped immediately between two large aluminum blocks precooled in liquid nitrogen. The frozen biopsies were divided into three approximately equal transmural sections: epicardium (identified by the methylene blue dye), midmyocardium, and endocardium. After the frozen sections were weighed, they were homogenized at 4°C in 6% perchloric acid using a Tekmar tissue homogenizer. Extracts were neutralized with 5 M K2CO3, and the supernatant was used for biochemical analyses that were performed at 340 nm on a Gilford 250 spectrophotometer (Oberlin, Ohio). An aliquot of neutralized extract was used in a coupled enzymatic reaction to determine phosphocreatine (PCr) and ATP. In a separate coupled reaction, ADP and AMP were determined. Tissue nucleotides were expressed as micromoles per gram dry weight, the total adenine nucleotide pool was calculated as the sum of ATP, ADP, and AMP content in each layer.

Tissue slices from normal and posts ischemic regions were weighed and dried to constant weight at 95°C in pretested vials. Total tissue water was expressed as milliliter H2O per 100 g dry tissue weight.

Experimental Protocol (See Figure 1)

After surgical preparation and stabilization of the animal, a pretreatment-control (PTC) measurement of hemodynamics, myocardial segment function, and blood flow was obtained. Radioactive microspheres were administered before saline or drug intervention. Saline (control series) or deferoxamine (500 mg) was administered as an intra-atrial infusion at 0.58 ml/min beginning 15 minutes prior to LAD occlusion and continued throughout the 15-minute ischemic period. Deferoxamine (deferoxamine mesylate, CIBA-Geigy) was dissolved in 0.9% saline immediately before use. Ten minutes after the infusion was begun, hemodynamics and myocardial segment function were determined. The LAD was then occluded for 15 minutes, during which time hemodynamics, myocardial segment shortening, and myocardial blood flow were determined. At the end of 15 minutes of ischemia, the occluder was slowly released allowing LAD coronary blood flow (CBF) to return to pretreatment values, that is, attenuating the reactive hyperemic response. Hemodynamics and myocardial function were determined at 5, 15, 30, 60, 120, and 180 minutes of reperfusion, while radioactive microspheres were administered at 30 and 180 minutes of reperfusion to determine regional myocardial blood flow.

\[ \text{FIGURE 1. Experimental protocol} \]

\( \text{(See text). Deferoxamine was infused intra-atrially (500 mg).} \) Hemodynamic and percent segment shortening measurements; Regional myocardial blood flow determinations by radioactive microspheres. CON, pretreatment control.
TABLE 1. Hemodynamics in Saline and Deferoxamine-Treated Dogs

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>MAP</th>
<th>LVSP</th>
<th>LVEDP</th>
<th>dP/dt mm Hg/s</th>
<th>CBF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pretreatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>146±4</td>
<td>96±4</td>
<td>108±4</td>
<td>3±1</td>
<td>2,432±136</td>
<td>24±3</td>
</tr>
<tr>
<td>DF</td>
<td>142±4</td>
<td>90±4</td>
<td>102±4</td>
<td>2±1</td>
<td>1,938±121</td>
<td>26±3</td>
</tr>
<tr>
<td>Post-Drug</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>146±4</td>
<td>96±4</td>
<td>107±4</td>
<td>3±1</td>
<td>2,470±139</td>
<td>25±3</td>
</tr>
<tr>
<td>DF</td>
<td>140±4</td>
<td>89±3</td>
<td>101±3</td>
<td>2±1</td>
<td>1,950±89</td>
<td>26±4</td>
</tr>
<tr>
<td>Occlusion 5 min</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>148±4</td>
<td>92±4</td>
<td>103±4</td>
<td>5±1</td>
<td>2,250±144</td>
<td></td>
</tr>
<tr>
<td>DF</td>
<td>140±5</td>
<td>81±5</td>
<td>92±5</td>
<td>3±1</td>
<td>1,890±133</td>
<td></td>
</tr>
<tr>
<td>30 minutes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>147±4</td>
<td>96±3</td>
<td>109±4</td>
<td>3±1</td>
<td>2,327±115</td>
<td>24±3</td>
</tr>
<tr>
<td>DF</td>
<td>143±5</td>
<td>88±6</td>
<td>100±6</td>
<td>2±1</td>
<td>1,988±121</td>
<td>27±3</td>
</tr>
<tr>
<td>60 minutes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>149±4</td>
<td>94±4</td>
<td>107±4</td>
<td>3±1</td>
<td>2,282±107</td>
<td>22±2</td>
</tr>
<tr>
<td>DF</td>
<td>146±5</td>
<td>92±7</td>
<td>107±6</td>
<td>2±1</td>
<td>2,250±109</td>
<td>27±4</td>
</tr>
<tr>
<td>180 minutes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>147±4</td>
<td>97±4</td>
<td>111±4</td>
<td>3±1</td>
<td>2,443±79</td>
<td>20±2</td>
</tr>
<tr>
<td>DF</td>
<td>141±4</td>
<td>89±7</td>
<td>104±6</td>
<td>2±1</td>
<td>2,145±103</td>
<td>24±4</td>
</tr>
</tbody>
</table>
| Values are mean±SEM (n = 14 control group; n = 10 deferoxamine group). CONTROL, saline-treated control group; DF, deferoxamine-treated group 500 mg; HR, heart rate; MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; CBF, coronary blood flow.

Statistical Analyses

All values are the mean±SEM. Hemodynamics were obtained from a mean of 3–5 cardiac cycles. Groups were compared using a two-way analysis of variance (ANOVA) with repeated measures design and Fisher's least-significant difference was used to test for the significance of difference between two groups at specific time points. When values were compared with the PTC, a Dunnett's t test was used. ANOVA followed by a paired t test was used to compare differences between adenine nucleotide and tissue water contents in nonischemic and ischemic-reperfused tissue. Means were considered significantly different if p<0.05.

Results

Of the 32 dogs initially instrumented, eight died of ventricular fibrillation immediately following reperfusion: seven of 21 in the control group and one of 11 in the deferoxamine group. The incidence of ventricular fibrillation after deferoxamine treatment was less than that in the control group, although this difference did not reach statistical significance. Analysis of data was thus performed for 14 control dogs and 10 deferoxamine-treated dogs.

Hemodynamics

The hemodynamics of both groups during pretreatment, at 12 minutes of occlusion, and at various times following reperfusion are summarized in Table 1. There were no significant differences in any hemodynamic parameter between control and deferoxamine-treated dogs at any time, nor were there any differences within any group throughout the experiment. The heart rate–left ventricular systolic pressure product, an indirect measure of myocardial oxygen demand was not different between the two groups prior to, during, or following the ischemic period (Figure 2).

Area at Risk

The left ventricular and area at risk weights were similar in both groups (left ventricular weight: control, 92±4; deferoxamine, 98±5 g; risk weight: control, 24±2; deferoxamine, 24±2 g). Thus, the percent of left ventricle at risk was also similar between groups (control, 27±2; deferoxamine, 25±2), demonstrating that equivalent perfusion bed areas were subjected to ischemia.

Regional Myocardial Blood Flow

Tissue blood flow within the normal, left circumflex-perfused bed was not different between the control and deferoxamine-treated animals throughout the experiment. Deferoxamine had no effect on blood flow to the nonischemic region in any myocardial layer. Transmural blood flow to the nonischemic zone was 0.95±0.14 in the control group and
0.77 ± 0.06 ml/min/g in deferoxamine-treated dogs. In the ischemic-reperfused region, no significant differences in blood flow (Figure 3), endo/epi ratio (during occlusion, control group = 0.60 ± 0.06; deferoxamine group = 0.62 ± 0.07), or the ratio of ischemic area transmural flow to nonischemic area transmural flow (during occlusion control = 0.20 ± 0.02; deferoxamine = 0.23 ± 0.05) were present before or during occlusion, thus demonstrating that both groups were subjected to comparable degrees of ischemia. During reperfusion (30 and 180 minutes), no differences were present in regional myocardial blood flow between the control and deferoxamine groups (Figure 3).

Myocardial Segment Shortening

There were no significant differences in normal area subendocardial wall function throughout the experiment in either control or deferoxamine groups (Figure 4). While %SS in the nonischemic region of the deferoxamine group tended to be greater than that in the control group, differences were not statistically significant (Baseline %SS: control group, 10.4 ± 0.7; deferoxamine group, 10.0 ± 1.2). Infusion of deferoxamine did not produce any change in %SS. Segment wall function in the ischemic-reperfused region is shown in Figure 5. Deferoxamine did not significantly influence %SS during the initial 15 minutes prior to the ischemic episode. %SS was similar in control and deferoxamine-treated animals prior to and during coronary occlusion. As expected, %SS rapidly decreased to nega-

![Figure 2. Heart rate-left ventricular systolic pressure product (HRxLVSP) in mm Hg · beats/min (10⁴) throughout the experimental time course in control and deferoxamine-treated animals. PTC, pretreatment-control; Drug, 10 minutes after drug infusion was begun; Occ, coronary occlusion.](image)

![Figure 3. Ischemic-reperfused region blood flow (ml/min/g) in subepicardium (EPI), midmyocardium, and subendocardium (ENDO) during pretreatment-control (control), coronary occlusion and 30 minutes and 3 hours postreperfusion in control and deferoxamine (DF)-treated groups. Values are mean±SEM.](image)

![Figure 4. Percent segment shortening (%SS) in the nonischemic area at pretreatment-control (PTC), 10 minutes after deferoxamine (DF) or saline infusion (control) was begun (Drug), 12 minutes of occlusion (Occ), and at various times following reperfusion. Each point is the mean±SEM. Absolute %SS values during PTC: control, 10.4 ± 0.7; DF, 10.0 ± 1.2%.](image)
Figure 5. Percent segment shortening (%SS) in the ischemic-reperfused region at pretreatment-control (PTC), 10 minutes after deferoxamine (DF) or saline infusion (control) was begun (Drug), 12 minutes of occlusion (Occ), and at various times following reperfusion. Each point is the mean ± SEM. *p < 0.05 versus control group. Absolute %SS values during PTC: control, 19.7 ± 1.6; DF, 5.5 ± 1.3.

Figure 6. ATP in reperfused zone expressed as a percentage of nonischemic zone concentration in epicardium (Epi), midmyocardium (Mid) and endocardium (Endo) for control and deferoxamine (DF) groups. *p < 0.05 vs. control group.

Figure 7. Phosphocreatine (PCr) in reperfused zone expressed as a percent of nonischemic zone concentration in epicardium (Epi), midmyocardium (Mid), and endocardium (Endo) for control and deferoxamine (DF) groups. *p < 0.05 vs. control group.

High-Energy Phosphates

Since mean transmural ATP and PCr in the nonischemic regions were similar between groups (ATP in control, 24.8 ± 0.6; in deferoxamine, 22.5 ± 0.5 μmol/g dry wt; PCr in control, 40.6 ± 1.5; in deferoxamine, 39.4 ± 1.3 μmol/g dry weight), ATP and PCr are expressed as a percent of the concentrations in the ischemic-reperfused area to concentrations in the nonischemic area and are illustrated in Figures 6 and 7, respectively. In general, ATP in the ischemic-reperfused zone was decreased and PCr increased in both groups. Endocardial ADP and AMP (not shown) were also decreased, and the changes from the nonischemic region were similar in control and deferoxamine groups. Thus, in general, both groups exhibited decreases in the total adenine nucleotide pool of the ischemic-reperfused region. However, in the deferoxamine group there was a significant preservation of ATP (Figure 6), and thus, total adenine nucleotides, specifically in the endocardial layer (percent of normal area ATP: control, 79 ± 3; deferoxamine, 93 ± 6%). In contrast to the significant reperfusion-induced rebound increase in PCr observed in all layers in the control group, there was not a signifi-
Aminé was concomitant with a significant preservation of endocardial ATP, a decrease in PCr rebound, and a reduction in myocardial tissue edema. These results strongly suggest that •OH radical or another radical derived from it, and/or iron-dependent lipid peroxidation play an important role in myocardial functional and metabolic stunning.

In addition to inhibiting iron-dependent lipid peroxidation and •OH formation by removing iron from oxidation-reduction cycling, deferoxamine has also demonstrated to directly scavenge •OH radicals. The affinity of deferoxamine for Fe³⁺ is extremely high (Kₐ = 10⁻³³) while the strength of chelation for other metal ions is several orders of magnitude lower. In vivo, the catalytic iron may be bound to proteins, membranes or chelating agents of low molecular weight. Some of these iron complexes have low binding constants (e.g., ADP and albumin) and may result in the formation of •OH. Low molecular–weight chelating agents (e.g., ATP and citrate) may also generate •OH, although they are only about 10% as effective as open-structured, iron-EDTA complexes. Also, the release of Fe³⁺ from lactoferrin has been shown to be essential for red blood cell lysis by neutrophils. Other possible sources for iron participating in •OH formation may be that loosely bound to carbohydrates and membrane lipids or tightly bound to myoglobin or cytochrome c.

The suggestion that oxygen-derived free radicals are involved in myocardial reperfusion damage has arisen from several models of ischemia-reperfusion, including infarct size, buffer-perfused isolated hearts, and stunned myocardium. Interventions used to inhibit free radicals in an attempt to decrease infarct size have been primarily aimed at preventing formation of •OH or scavenging •OH radicals. Treatment with allopurinol, N-2-mercaptopyrropropionyl glycine and superoxide dismutase + catalase prior to occlusion have each been shown to decrease myocardial infarct size after a 90-minute coronary occlusion in dogs without any effect on myocardial oxygen supply-demand. In contrast, others have demonstrated a lack of benefit on infarct size with allopurinol and with superoxide dismutase + catalase. Differences between these studies are not readily apparent but may involve measurement of coronary blood flow, triphenyltetrazolium chloride staining versus histology to measure infarct size, or different durations of occlusion and reperfusion. Thus, the role of oxygen-derived free radicals as mediators of progressive irreversible myocardial damage during reperfusion remains unclear at the present time.

In buffer-perfused, globally ischemic, isolated heart preparations, several studies have demonstrated cardioprotective effects of agents that decrease free radicals. Preventing formation of •OH radical by iron chelation has been suggested by Menasche and coworkers as an important modification of cardioprotection. In rat hearts subjected to 3 hours of hypothermic cardioprotective arrest, followed by 45 minutes of reperfusion, deferox-
amine exhibited marked cardioprotective effects on pressure development, ventricular dP/dt, left ventricular compliance, and coronary blood flow during reperfusion. Using isolated rabbit hearts in a model of cardioplegic arrest, Myers et al. showed that deferoxamine treatment with cardioplegia increased posts ischemic coronary blood flow without any influence on contractile recovery. One possible explanation for this lack of effect on contractile force may have been the relatively small concentrations of deferoxamine used in this study as compared with those of Menasche et al. who suggested a strong dose-response relation. An enhancement of posts ischemic myocardial function and metabolism, even when deferoxamine was given just prior to reperfusion, has also been shown in isolated Langendorff-perfused rabbit hearts subjected to 30 minutes of normothermic global ischemia and 45 minutes of reperfusion. Deferoxamine-treated hearts recovered developed pressure, intracellular pH, and PCr content to a greater extent than control hearts. These findings lend further support to the concept of •OH-induced myocardial damage during long term, global ischemia.

In the stunned myocardium, several agents (e.g., superoxide dismutase + catalase) that are known to scavenge •O₂⁻ and H₂O₂ have been administered prior to a 15-minute coronary occlusion and have shown similar beneficial effects on regional posts ischemic functional recovery. More recently, Bolli et al. administered the •OH radical scavenger, dimethylthiourea, and demonstrated significant enhancement of regional myocardial function following a brief ischemic episode. If, as suggested by Girotti and Halliwell and Gutteridge, the formation of •OH is "site specific" and dependent upon the location of the metal catalyst for the reaction, •OH scavengers may not necessarily act in vivo as expected from the chemically determined rate constants obtained from in vitro studies. The action of the scavenger depends on it being equally accessible to the •OH radical as the target molecule as well as its ability to enter specific microenvironments where the radicals may be formed. Thus, •OH radicals may participate in the initiation of lipid peroxidation in liposomal or microsomal fractions even though •OH scavengers do not inhibit iron-stimulated peroxidation. For this reason, the method of iron chelation, rather than direct scavenging of •OH radical alone, was chosen in the present study to determine the role of iron and consequently the •OH radical in the stunned myocardium.

The reason(s) for the prolonged abnormalities observed in the stunned myocardium in the absence of cellular necrosis is still unknown. It has been suggested that the primary cause of this phenomenon after a 15-minute coronary occlusion is neutrophils or neutrophil-derived superoxide anion free radicals. In that study, neutrophils were depleted by use of leukopak filters. This procedure also activated the complement system, decreased platelet counts and caused release of adenosine. As a vasodilator may increase blood flow to the ischemic region and in this way enhance myocardial function. While controversy exists on the importance of neutrophils after brief episodes of ischemia, deferoxamine should have potential to decrease neutrophil-mediated free radical damage if it occurs.

Other mechanisms for the prolonged abnormalities observed after a brief occlusion and reperfusion have been postulated. The stunned myocardium appears to retain considerable energy reserve that may be mobilized by positive inotropic stimulation by epinephrine and postextrasystolic potentiation. Thus, dysfunction may involve an uncoupling of contraction with ATP utilization rather than a deficient energy store. While a poor correlation between recovery of ATP during reperfusion and myocardial function has been observed, a decreased coupling of myofibrillar creatine kinase and ATPase has been suggested as a possible mechanism responsible for functional abnormalities found in stunning. If free ADP concentrations are below the Kₐ for myofibrillar creatine kinase, it is possible that the deferoxamine-related increase in adenine nucleotide levels found in the present study either yielded higher free ADP concentrations for creatine kinase or increased the amount of localized ATP for myosin ATPase.

Although the mechanism(s) responsible for the preservation of endocardial ATP after deferoxamine administration is unknown, possibilities include an attenuation of the fall in ATP during ischemia or an increase in its production during reperfusion. During coronary occlusion, no differences were observed in the degree of ischemia as evidenced by the loss of systolic function and levels of collateral blood flow; therefore, it is unlikely that ATP concentrations were greater during occlusion in the deferoxamine-treated group. In this regard, the study by Ambrosio and colleagues also showed evidence by use of ³¹P NMR of an increase in posts ischemic myocardial ΔATP from 35 ±6% to 51 ±6% of preischemic levels by deferoxamine treatment prior to reperfusion. Although this was not statistically significant, the magnitude of this increase was approximately 46%. This suggests a component of ATP preservation perhaps unrelated to changes occurring during the ischemic period. In contrast, oxygen free radicals, particularly •OH, may alter membrane integrity by causing membrane lipid peroxidation, by degrading hyaluronic acid (membrane and glycocalyx component), or by promoting neutrophil accumulation, adhesion and activation. However, a role for •OH radical in lipid peroxidation has been refuted by lack of both theoretical as well as experimental evidence. By whatever mechanism, a deferoxamine-related decrease in free radicals, may preserve membrane integrity and allow...
less utilization of high-energy phosphates for purposes of maintaining ionic homeostasis. In addition, free radicals may alter the activity and function of certain membrane-bound enzymes such as Na⁺K⁺-ATPase.⁴¹ If the disruption in myofibrillar creatine kinase observed by Greenfield and Swain²⁸ is also, in part, due to free radicals, deferoxamine could preserve enzyme activity, allowing a greater shunting from PCr to ATP in response to an increase in myocardial function and an enhanced rate of ATP utilization. This would also explain the attenuation of the PCr rebound in the deferoxamine-treated group.

Preventing free radical-induced membrane damage may also explain the present finding of decreased tissue edema after deferoxamine treatment. This observation is in agreement with that of Korthuis et al⁴² who observed that oxygen radicals were the primary cause of an increase in skeletal muscle vascular permeability produced by ischemia-reperfusion. Further, these authors suggest the OH radical as the primary damaging species. Also, generation of oxygen free radicals by electrolysis of a buffer solution and subsequent perfusion through nonischemic isolated rabbit hearts, lead to an accumulation of ¹²⁵I-labeled albumin, indicative of an increased vascular permeability.⁴³ Associated with this increased permeability, were an increase in coronary artery perfusion pressure and left ventricular end-diastolic pressure, which, along with the permeability changes, were also prevented by inhibition of free radicals.⁴⁴ When oxygen free radicals were generated via a purine-xanthine oxidase system a decrease in the peak rate and magnitude of force generation in rat papillary muscle was observed.⁴⁴ These results strongly suggest that oxygen free radicals may, even in the absence of ischemic damage, impair myocardial function, and that the beneficial effect of deferoxamine on functional recovery in the present study may involve inhibition of a direct stunning action by free radicals during reperfusion. That free radicals caused a decrease in the rate of force development⁴⁴ may be related to the shift in extracellular Ca²⁺ sensitivity observed in stunned ferret hearts.⁴⁵ The possibility exists that sarcoplasmic reticular–membrane disruption by free radicals decreases Ca²⁺ transients, subsequently depresses Ca²⁺ sensitivity⁴⁵,⁴⁶ and results in abnormal contractility.

In conclusion, the pathophysiological role of iron, and thus iron-mediated hydroxyl radical formation and lipid peroxidation in the stunned myocardium has been clearly delineated. Administration of the iron-chelating agent, deferoxamine, results in significant attenuation of both myocardial dysfunction and metabolic abnormalities as well as a decrease in tissue edema following 15 minutes of coronary occlusion and 3 hours of reperfusion in the absence of changes in classic myocardial oxygen supply-demand parameters.

Note added in proof: Recently, Bolli and coworkers have also demonstrated that deferoxamine attenuates postischemic dysfunction in a canine model similar to the one used in the present study. These data have been published in Am J Physiol 1987;253: H372–H380.

Acknowledgments

The authors wish to acknowledge Ms. Anna Hsu, Ms. Jeannine Moore, and Ms. Susan Fromm for their excellent technical assistance.

References

6. Reimer KA, Jennings RB: Failure of the xanthine oxidase inhibitor allopurinol to limit infarct size after ischemia and reperfusion in dogs. Circulation 1985;71:1069–1075
8. Richard VR, Murry CE, Jennings RB, Reimer KA: Superoxide dismutase and catalase do not limit infarct size after 90 minutes of ischemia and 4 days of reperfusion in dogs (abstract). Circulation 1987;76:199
tdoxyl free radicals: Cause of ischemia-
29. Ambrosio G, Zweier JL, Jacobus WE, Weisfeldt ML, Fla-
37. Hoffman HJ, Mauser M, Schaper W: Effect of adeno-
38. Greenfield RA, Swain JL: Disruption of myofibrillar energy use: Dual mechanisms that may contribute to postischemic dysfunction in stunned myocardium. Circ Res 1987;60:283-289
41. Kim MS, Akera T: O2 free radicals: Cause of ischemia-
46. Rowe GT, Manson NH, Coplan M, Hess ML: Hydrogen peroxide and hydroxyl radical mediation of activated leu-
cyte depression of cardiac sarcoplasmic reticulum. Circ Res 1983;53:584-591

KEY WORDS • regional myocardial function • deferoxamine • ischemia • blood flow, regional myocardial • coronary reperfusion • adenine nucleotides • iron
Evidence for a role of iron-catalyzed oxidants in functional and metabolic stunning in the canine heart.

N E Farber, G M Vercellotti, H S Jacob, G M Pieper and G J Gross

doi: 10.1161/01.RES.63.2.351

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Copyright © 1988 American Heart Association, Inc. All rights reserved.

Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:

http://circres.ahajournals.org/content/63/2/351

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Circulation Research* is online at:
http://circres.ahajournals.org/subscriptions/