Superoxide Dismutase Reduces Reperfusion Arrhythmias but Fails to Salvage Regional Function or Myocardium at Risk in Conscious Dogs

Jun Nejima, MD, Delvin R. Knight, PhD, John T. Fallon, MD, PhD,
Nobuhisa Uemura, MD, W. Thomas Manders, BA, Don R. Canfield, DVM,
Michael V. Cohen, MD, and Stephen F. Vatner, MD

To determine if oxygen free radical scavengers administered before coronary artery reperfusion can limit reperfusion arrhythmias, increase the return of regional function in ischemic myocardium, and reduce tissue necrosis at 1 week after 90-minute coronary artery occlusion and reperfusion, conscious dogs were treated with superoxide dismutase (SOD) and catalase before and for 1 hour after coronary artery reperfusion. Another group was treated with recombinant SOD (rSOD) because the commercially available SOD and catalase contained endotoxin. The conscious dogs were studied 3–4 weeks after implanting left ventricular pressure gauges, ultrasonic wall thickness gauges in the posterior left ventricular wall, left atrial catheters, and arterial catheters, Doppler flow transducers, and hydraulic occluders on the left circumflex coronary artery. The only beneficial effect observed was that the number of arrhythmic beats per minute in the rSOD-treated group was significantly lower (p < 0.05) when compared with a control group after coronary artery reperfusion. Treatment neither increased the amount of recovery of wall thickening in the ischemic zone nor reduced infarct size when expressed either as a percentage of the area at risk or as a function of collateral blood flow in the ischemic zone. For example, infarct size as a percentage of the area at risk was 32.6±5.8%, 37.4±6.4%, 28.3±5.1% in the control, SOD and catalase–, and rSOD-treated groups, respectively. Thus, although treatment with oxygen-free radical scavengers invoked a transient reduction in the number of reperfusion arrhythmias, this treatment in conscious dogs failed to improve regional myocardial dysfunction or reduce the amount of necrosis when compared with a control group. The lack of a sustained salutary effect may indicate that longer periods of treatment with free radical scavengers are required in chronic preparations. (Circulation 1989;79:143–153)

Coronary artery reperfusion is used with increasing frequency in the therapy of acute myocardial infarction. However, the relatively short time of coronary artery occlusion after which reperfusion can no longer salvage myocardial tissue and ischemic myocardial function limits the usefulness of this intervention. For example, studies in experimental models suggest that after 3 hours of coronary artery occlusion, acute reperfusion results in relatively little salvage of myocardial function or tissue at risk.1–4 The results from these studies, that is, the relatively short time period that reperfusion can elicit beneficial effects, make the concept of reperfusion damage particularly attractive. The concept of reperfusion damage suggests that a major contributor to myocardial necrosis is the reperfusion, that is, a sequence of biochemical events tied to the rapid reoxygenation of ischemic myocardium.5–8 One of the popular mechanisms thought to mediate reperfusion damage involves oxygen free radicals, which can be made ineffective
by administration of oxygen free radical scavengers before coronary artery reperfusion.9-11

The goal of this investigation was to determine if administration of the oxygen free radical scavenger, superoxide dismutase (SOD), just before and immediately after coronary artery reperfusion can limit the appearance of reperfusion arrhythmias, enhance the return of regional function in ischemic myocardium, and reduce the size of myocardial infarction after 90 minutes of coronary artery occlusion and reperfusion in conscious dogs. The period of 90 minutes of coronary artery occlusion was selected because this time of ischemia has been shown to be particularly optimal in establishing a beneficial effect of SOD in acutely prepared anesthetized animals.8-10 Four groups of animals were studied: two saline control groups, one with and one without lidocaine and two treated groups, one treated with commercially available SOD and catalase (CAT) and one treated with human recombinant superoxide dismutase (rSOD). The two untreated control groups were examined separately because lidocaine may affect oxygen free radicals through its effects on leukocytes.12-14 The two treated groups were examined separately because the commercially available SOD and CAT were found to contain endotoxin.

Materials and Methods

Animal Preparation

Thirty-nine mongrel dogs of either sex, after preanesthesia with xylazine (0.5 mg/kg) and general anesthesia with sodium pentobarbital (30 mg/kg) had instruments implanted in preparation for the experiment. Under sterile conditions, the chest was opened at the left fifth intercostal space, the pericardium incised, and the heart exposed. Tygon catheters were implanted in the left atrium and descending aorta for measurement of atrial and arterial pressures and for the injection of microspheres and withdrawal of reference blood samples to measure regional myocardial blood flow. A solid-state miniature pressure gauge (Konigsberg Instruments, Pasadena, California) was implanted in the left ventricular cavity for measurement of left ventricular pressure. The left circumflex coronary artery was carefully dissected, and a hydraulic occluder and an ultrasonic Doppler blood flow transducer were implanted to occlude and reperfuse the vessels and to verify complete occlusion and reperfusion, respectively. The potential ischemic area was then delineated by occluding the artery for approximately 30 seconds. A pair of ultrasonic crystals to measure wall thickness was implanted across the left ventricular free wall in the center of the area that became ischemic. The chest was closed and evacuated, and the lead wires and catheters were tunneled subcutaneously to the interscapular region. All animals were allowed 3–4 weeks to recover from surgery. At that time, the animals were vigorous and healthy and had normal body temperatures (102.0±0.1°F), hematocrit level (47±1.7%) and white blood cell counts (13,825±878 counts/cm³).15 Animals used in this study were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and with those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHHS Publication No. [NIH] 85-23, revised 1985).

Measurements

Left ventricular pressure was measured with the implanted miniature pressure gauge, which was calibrated in vitro as well as in vivo during the experiments by cross calibrating this measurement with the systolic arterial pressure measurement with use of Statham P23ID strain gauge manometers (Cleveland, Ohio). The first derivative of left ventricular pressure, left ventricular dP/dt, was obtained with an operational amplifier connected as a differentiator, which has a frequency response of 700 Hz. Arrhythmias, as reflected in lead II of the electrocardiogram (ECG), were quantified by counting all abnormal beats for 10 minutes at each data point.

Regional myocardial function was measured with an ultrasonic transit time dimension gauge previously described.3,4 The instrument measures the transit time of acoustic signals traveling at a sonic velocity of 1.58×10⁶ mm/sec between two intramyocardial crystals. The drift of this instrument, although minimal (less than 0.01 mm/6 hr), was effectively eliminated by repeated calibrations throughout the experiment. The radioactive microsphere technique, as previously described by Domenech et al16 and used in this laboratory,3,17,18 was used to measure regional myocardial blood flow. In this study, 2 to 3 million (15±1 μm) microspheres labeled with ¹⁴Ce, ⁵¹Cr, ⁹⁹Nb, ⁸⁵Sr, ⁴⁸Sc, ⁷⁵Se, ⁹⁰Ru, ¹⁰³Sn, or ¹¹¹In were used (New England Nuclear, Boston, Massachusetts). These microspheres were suspended in 0.01% monooate (Tweem 80) solution (10% dextran) and placed in an ultrasonic bath for 60 minutes. Before the first injection of microspheres, 1.0 ml Tweem 80 solution was injected to test for potential adverse cardiovascular effects.¹⁹ Microspheres were injected and flushed with 10 ml saline during a 20-second period through the left atrial catheter. Arterial blood reference samples were withdrawn at a rate of 7.75 ml/min for a total of 120 seconds beginning 30 seconds before microsphere injection. Because these experiments included periodic measurements for 1 week after coronary artery occlusion, a correction factor for blood flow in the ischemic myocardium was used to minimize error due to a potential “microsphere loss.”⁵¹,²⁰,²¹ To correct for microsphere loss, individual values for blood flow in the ischemic tissue were multiplied by the ratio of average blood flow in the nonischemic myocardium to blood flow in the ischemic myocardium measured before coronary artery occlusion. For
blood flow measurements, the myocardial samples were divided into three layers (endocardium, midwall, and epicardium), weighed, and counted for 5 minutes in a multichannel gamma counter with a germanium well detector (Canberra, Meriden, Connecticut) with appropriately selected energy windows. After correcting the counts for background and crossover, regional myocardial blood flow was calculated and expressed as milliliters per minute per gram of tissue.

Protocol

Experiments were performed 3–4 weeks after recovery from surgery. With the dogs lying quietly on their right side, recordings of arterial pressure, left ventricular systolic pressure, left ventricular dp/dt, heart rate, electrocardiogram (lead II), and posterior left ventricular wall thickening (ischemic zone) were obtained during the baseline period, after premedication with morphine (0.3 mg/kg i.m.), continuously throughout the coronary artery occlusion, and for the first 3 hours after reperfusion. Hemodynamic measurements were recorded again at 1, 2, and 4 days and 1 week after coronary artery reperfusion. After baseline measurement of myocardial blood flow was also obtained by injection of radioactive microspheres, coronary artery occlusion was accomplished by inflating the hydraulic occluder. Complete coronary artery occlusion was confirmed during the experiment by complete loss of systolic wall thickening in the ischemic zone and loss of the Doppler flow signal. Coronary artery occlusion was later verified by a marked decrease in blood flow in the ischemic area as measured by the radioactive microsphere technique. Microspheres were injected for the second time at 5 minutes after coronary artery occlusion. Potentially lethal, ventricular premature contractions and tachycardia were treated with 2% lidocaine (20 mg/ml i.v.) during the first hour of coronary artery occlusion. During this time period, the three groups received similar total amounts of lidocaine: 2.1 ± 0.8 ml in the control group, 3.1 ± 1.7 ml in the SOD and CAT group, and 3.3 ± 1.6 ml in the rSOD group. Microspheres were injected for a third time at 85 minutes after coronary artery occlusion. The occluder was gradually deflated during an approximately 1-minute period after 90 minutes of coronary artery occlusion. Microspheres were also injected 5 minutes, 3 hours, and 24 hours after coronary artery reperfusion. At 5 minutes before coronary artery reperfusion, one group (SOD and CAT, n = 8) received 2 ml 2% lidocaine (left atrial bolus), SOD (Sigma Chemical, St. Louis, Missouri) 6.5 mg/kg (left atrial bolus) followed by 0.4 mg/kg/min (left atrial infusion) for 15 minutes followed by 0.08 mg/kg/min (left atrial infusion) for 45 minutes, and CAT (Sigma Chemical) 0.2 mg/kg/min (left atrial infusion) for 15 minutes followed by 0.04 mg/kg/min (left atrial infusion) for 45 minutes. At 5 minutes before coronary artery reperfusion, another group (rSOD, n = 10) received 2 ml 2% lidocaine (left atrial bolus), human rSOD (Chiron and Pharmacia, Piscataway, New Jersey), 6.5 mg/kg (left atrial bolus) followed by 0.4 mg/kg/min (left atrial infusion) for 15 minutes followed by 0.08 mg/kg/min (left atrial infusion) for 45 minutes. Another group (control group that received lidocaine, n = 9) received 2 ml 2% lidocaine before reperfusion and saline solution, which was administered in identical amounts and manner as in the treated groups. A fourth group (control, with no lidocaine, n = 6) received saline, but no lidocaine was administered at any time. When the data from the latter two groups were combined, the group was identified as the combined control group. To determine whether or not the SOD and CAT were contaminated with endotoxin, samples of these compounds were tested with the Limulus Amebocite Lysate test (Associate of Cape Cod, Woods Hole, Massachusetts). The SOD (Sigma Chemical) and CAT (Sigma Chemical) were found to contain 250–2,500 units/mg endotoxin; endotoxin was not detectable in rSOD. Six of eight dogs receiving the commercially available SOD and CAT developed bloody diarrhea; this symptom was not observed in any of the other animals in the study. Because of this finding, the study was conducted in the following manner. Initially, five control and five SOD and CAT–treated dogs were studied randomly. Then four control with lidocaine, four control without lidocaine, three SOD and CAT–treated, and 10 rSOD-treated dogs were added. Then, two control dogs without lidocaine were added. Accordingly, 33 dogs (nine control with lidocaine, six control without lidocaine, eight SOD and CAT–treated, and 10 rSOD-treated) were enrolled in the data analysis.

Serial samples of blood were withdrawn from the animals during the 1-week period. To determine whether or not there were appropriate blood levels of rSOD during coronary artery reperfusion, plasma levels of rSOD were obtained before occlusion; 1 hour after occlusion; 5, 15, and 30 minutes after coronary artery reperfusion; and 1, 3, 4, 5, 6, and 12 hours, 1 and 2 days, and 1 week after coronary artery occlusion (Figure 1). Plasma levels of rSOD reached 356 ± 14 μg/ml at 15 minutes after coronary artery reperfusion and was maintained at more than 100 μg/ml until 1 hour after reperfusion. The samples for plasma creatine kinase measurement were taken every hour for 8 hours, every 2 hours for the next 8 hours, and every 4 hours for the final 8 hours. The samples were collected in tubes containing ethyleneglycol-bis-(β-aminoethyl ether)-N,N',N''-tetraacetic acid (EGTA) and centrifuged. The plasma was decanted and frozen immediately at −70° C. Creatine kinase in plasma was assayed spectrophotometrically as described by Rosalki.

Pathology

After 1 week, the dogs were anesthetized with sodium pentobarbital, injected with heparin, and sacrificed with a lethal dose of potassium chloride.
The heart was removed and placed in a perfusion apparatus for dual perfusion of the coronary circulation. The ascending aorta was perfused retrogradely with Evans blue, 1 mg/ml saline; the left circumflex coronary artery was cannulated at the site of occlusion and perfused at the same pressure with saline. Patency of the occluded coronary artery was verified and the heart was placed in 5% buffered formalin for 2 days.

The ventricles were sliced into multiple, 4 mm-thick rings and weighed, and the basilar side of each ring was photographed. One ring from each heart that contained the infarct was processed in its entirety for light microscopy. The infarct size was calculated by planimetry of the epicardium, ventricular cavities, and infarct borders from photographs of individual ventricular rings (magnification, \( \times 2.5 \)).

The pathology was conducted without knowledge of the treatment group. The area at risk was determined by planimetry of the zone that did not stain after infusion with Evans blue. In the two dogs that died at 10 and 15 hours after reperfusion, the areas at risk of infarction were determined with a myocardial blood flow technique\(^\text{18}\) and the triphenyl tetrazolium chloride technique,\(^\text{24}\) respectively. The blood flow technique involved calculation of area at risk by measurement of blood flow in all pieces of myocardium. Reductions of blood flow greater than 85% were assumed to indicate myocardium totally at risk. Blood flow reductions of 25–85% were considered to be proportionately less at risk. The infarcts were compared among groups as a function of area at risk and also as a function of collateral blood flow.\(^\text{2,25}\)

**Data Analysis**

All signals were recorded and stored on magnetic tape during experiments with a fourteen-channel tape recorder and played back on two multichannel direct-writing oscillographs displaying 16 simultaneous channels of data. Mean values and standard errors were calculated with an IBM PC/AT computer. Significant changes from preocclusion values were evaluated by Student’s \( t \) test for paired data and Bonferroni’s correction for multiple comparisons.\(^\text{26}\)

Significant changes between the control groups and the rSOD-treated group and between the control groups and the SOD and CAT-treated group were evaluated by one-way analysis of variance and a Student-Newman-Keuls test.\(^\text{27}\)

The correlation of collateral blood flow and infarct size was assessed by linear regression analysis.

**Results**

**Ventricular Fibrillation**

Experiments were initiated in 39 dogs. During coronary artery occlusion, six dogs developed ventricular fibrillation, only one of which was converted to sinus rhythm by direct current shock treatment. That dog was in the rSOD group. Of the 34 dogs that survived, six dogs developed ventricular fibrillation after coronary artery reperfusion, (three in control, one in SOD and CAT–treated, and two in the rSOD-treated group). Three of these six dogs were converted to sinus rhythm (one in the control group, two in the rSOD group). Of the three remaining dogs that had fibrillation after coronary artery reperfusion, one dog in the control group that died within 3 hours after reperfusion was excluded because we were unable to measure infarct size accurately at that time point. The remaining two dogs, in the SOD and CAT–treated and control groups, that died at 10 and 15 hours after reperfusion, respectively, were included. Therefore, although 31 dogs survived for 1 week (14 in control, seven in SOD and CAT–treated, and 10 in rSOD-treated group), the number of dogs included in the study was 33 (15 in control, eight in SOD and CAT–treated, and 10 in the rSOD-treated group).

**Pathology**

The area at risk, the absolute size of infarct, and infarct size expressed as a percentage of area at risk were not significantly different between the control group receiving lidocaine and the control group that did not receive lidocaine (Figure 2 and Table 1). Infarct size, expressed as a percentage of the area at risk, was also similar in the combined control group (32.6 ± 5.8%), SOD and CAT–treated group (37.4 ± 6.4%), and rSOD-treated group (28.3 ± 5.1%).

Infarct size, expressed as a percentage of area at risk and plotted as a function of transmural collateral flow (normalized for transmural blood flow in the nonischemic zone), did not indicate any significant difference among the treated groups or the control groups in either the control subgroup of nine dogs receiving lidocaine or the 15 dogs in the combined control groups (Figure 3). The equations for these regressions were control subgroup with lidocaine, \( y = -226x + 53, r = 0.62 \); combined control group \( y = -235x + 56, r = 0.69 \); SOD and CAT–treated...
group, $y = -258x + 60$, $r=0.92$; and rSOD-treated group, $y = -162x + 46$, $r=0.80$. The difference of the regression among the treated and control groups was not significant ($F=0.47$).

To determine if there was a difference in the effect of treatment in animals with less collateral blood flow, the infarct size as a percentage of area at risk was examined with a collateral blood flow index of less than 0.10 and greater than 0.10 (Figure 4). With use of this type of analysis, infarct size was not different among the combined control group and the treated groups in the animals with either low or high collateral blood flow.

**Hemodynamic Measurements**

The effect of coronary artery occlusion for 90 minutes and coronary artery reperfusion for 1 week on mean arterial pressure, heart rate, left ventricular systolic pressure, left ventricular dP/dt, mean left atrial pressure, and systolic wall thickening in the ischemic zone are shown in Table 2. Coronary artery occlusion did not affect left ventricular systolic pressure, mean arterial pressure, or left ventricular dP/dt significantly. Early after reperfusion, mean arterial pressure and left ventricular systolic pressure tended to fall in the SOD and CAT–treated and rSOD-treated groups. However, these changes were not significantly different from those in the control groups. At 1 week after coronary artery reperfusion, left ventricular systolic pressure, mean arterial pressure, and left ventricular dP/dt were not different from baseline values before coronary artery occlusion. Heart rate rose in all groups after coronary artery occlusion and remained elevated for the next 2 days. Mean left atrial pressure rose early after coronary artery occlusion but was no longer significantly elevated by 90 minutes after coronary artery occlusion. Wall thickening in the ischemic zone was reduced by more than 100% (i.e., paradoxical wall thinning was observed in all groups after coronary artery occlusion) (Figure 5). During the next week, partial recovery of systolic wall

**Table 1. Pathology**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Body weight (kg)</th>
<th>LV+RV weight (g)</th>
<th>Infarct weight (g)</th>
<th>Area at risk (g)</th>
<th>% INF/AAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined control group</td>
<td>15</td>
<td>20.6±0.6</td>
<td>143.2±5.7</td>
<td>11.8±2.4</td>
<td>34.3±2.1</td>
<td>32.6±5.8</td>
</tr>
<tr>
<td>Control group with lidocaine</td>
<td>9</td>
<td>20.1±0.9</td>
<td>137.4±8.4</td>
<td>10.9±3.0</td>
<td>33.7±2.1</td>
<td>32.2±8.1</td>
</tr>
<tr>
<td>Control group without lidocaine</td>
<td>6</td>
<td>21.5±0.7</td>
<td>151.9±5.9</td>
<td>13.2±4.4</td>
<td>35.2±4.4</td>
<td>33.2±8.7</td>
</tr>
<tr>
<td>SOD+CAT group</td>
<td>8</td>
<td>21.1±0.8</td>
<td>145.0±11.4</td>
<td>20.2±5.5</td>
<td>47.8±7.5</td>
<td>37.4±6.4</td>
</tr>
<tr>
<td>rSOD group</td>
<td>10</td>
<td>22.7±0.6</td>
<td>159.7±6.0</td>
<td>12.0±2.6</td>
<td>39.5±4.4</td>
<td>28.3±5.1</td>
</tr>
</tbody>
</table>

LV, left ventricle; RV, right ventricle; INF, infarct size; AAR, area at risk; SOD+CAT, superoxide dismutase and catalase; rSOD, recombinant superoxide dismutase.

No significant differences were observed among the groups.
thickening was observed in all groups. There was no significant difference in recovery of systolic wall thickening among the combined control group and the treated groups (Figure 5).

**Regional Myocardial Blood Flow**

In the nonischemic zone, epicardial and endocardial blood flow rose slightly after coronary artery occlusion in all groups. There was no difference in the blood flow responses to coronary artery occlusion or coronary artery reperfusion among the groups, except for the one point noted in Table 3.

In the ischemic zone, blood flow fell to minimum values in the endocardial and epicardial layers immediately after coronary artery occlusion and, by 85 minutes after coronary artery occlusion, increased in all groups. At this time, just before treatment, there was no difference among the groups for endocardial blood flow and epicardial blood flow. Early after reperfusion, blood flow rose above control levels in all three groups (Figure 6). The only significant difference in blood flow in the ischemic zone was in the control group without lidocaine at 3 hours after reperfusion as noted in Table 3.

**Arrhythmias**

For these analyses, the dogs in the control subgroup that received no lidocaine were excluded. There was no difference in the number of arrhythmic beats per minute in the three groups during the control period or 30, 60, and 90 minutes after coronary artery occlusion. During the initial reperfusion period, the number of arrhythmic beats increased significantly in all three groups. However, the number of arrhythmic beats per minute in the rSOD-treated group was lower (*p < 0.05*) than that observed in either the control group or the SOD and CAT-treated group at 2 hours after coronary artery reperfusion. Between 3 hours and 1 week after reperfusion, there was no difference in the arrhythmic beats per minute in either of the treated

### Table 2. Hemodynamic Measurements

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Coronary occlusion</th>
<th>Coronary reperfusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 min</td>
<td>90 min</td>
</tr>
<tr>
<td>Mean arterial pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 9)</td>
<td>97±3.5</td>
<td>114±8.1</td>
<td>98±6.0</td>
</tr>
<tr>
<td>No lidocaine (n = 6)</td>
<td>102±4.8</td>
<td>114±6.1</td>
<td>102±5.3</td>
</tr>
<tr>
<td>SOD+CAT (n = 8)</td>
<td>101±4.3</td>
<td>105±4.3</td>
<td>107±3.0</td>
</tr>
<tr>
<td>rSOD (n = 10)</td>
<td>97±4.6</td>
<td>109±2.9</td>
<td>101±4.0</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 9)</td>
<td>93±5.0</td>
<td>121±4.2</td>
<td>116±5.9</td>
</tr>
<tr>
<td>No lidocaine (n = 6)</td>
<td>84±4.7</td>
<td>124±9.6</td>
<td>112±9.4</td>
</tr>
<tr>
<td>SOD+CAT (n = 8)</td>
<td>85±6.0</td>
<td>119±12.7</td>
<td>123±9.0</td>
</tr>
<tr>
<td>rSOD (n = 10)</td>
<td>81±5.8</td>
<td>124±9.0</td>
<td>107±4.2</td>
</tr>
<tr>
<td>Left ventricular systolic pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 9)</td>
<td>124±4.5</td>
<td>128±9.3</td>
<td>119±7.5</td>
</tr>
<tr>
<td>No lidocaine (n = 6)</td>
<td>129±6.7</td>
<td>134±9.5</td>
<td>121±7.9</td>
</tr>
<tr>
<td>SOD+CAT (n = 7)</td>
<td>122±4.6</td>
<td>118±5.1</td>
<td>122±3.7</td>
</tr>
<tr>
<td>rSOD (n = 10)</td>
<td>120±4.8</td>
<td>123±3.1</td>
<td>120±4.9</td>
</tr>
<tr>
<td>Mean left atrial pressure (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 7)</td>
<td>2.6±0.8</td>
<td>10.7±3.7</td>
<td>4.8±1.1</td>
</tr>
<tr>
<td>No lidocaine (n = 5)</td>
<td>6.4±0.8</td>
<td>12.9±2.1</td>
<td>8.7±2.9</td>
</tr>
<tr>
<td>SOD+CAT (n = 8)</td>
<td>4.7±0.9</td>
<td>10.7±1.7</td>
<td>6.4±1.0</td>
</tr>
<tr>
<td>rSOD (n = 10)</td>
<td>4.4±1.0</td>
<td>9.7±1.8</td>
<td>6.5±1.2</td>
</tr>
<tr>
<td>Ischemic zone wall thickening (mm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n = 9)</td>
<td>2.80±0.33</td>
<td>-0.40±0.20</td>
<td>-0.06±0.16</td>
</tr>
<tr>
<td>No lidocaine (n = 5)</td>
<td>1.94±0.29</td>
<td>-0.19±0.20</td>
<td>0.09±0.12</td>
</tr>
<tr>
<td>SOD+CAT (n = 8)</td>
<td>3.91±0.39</td>
<td>-0.41±0.24</td>
<td>-0.32±0.28</td>
</tr>
<tr>
<td>rSOD (n = 10)</td>
<td>2.63±0.39</td>
<td>-0.53±0.16</td>
<td>-0.13±0.16</td>
</tr>
</tbody>
</table>

SOD+CAT, superoxide dismutase and catalase; rSOD, recombinant superoxide dismutase.

No significant differences were observed among the groups.
groups when compared with the control subgroup receiving lidocaine (Figure 7).

**TABLE 3. Regional Blood Flow**

<table>
<thead>
<tr>
<th></th>
<th>Nonischemic zone</th>
<th>Ischemic zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Coronary artery occlusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 min</td>
</tr>
<tr>
<td>Endocardial</td>
<td>Control (n=9)</td>
<td>1.05±0.11</td>
</tr>
<tr>
<td></td>
<td>No lidocaine (n=6)</td>
<td>1.11±0.14</td>
</tr>
<tr>
<td></td>
<td>SOD + CAT (n=8)</td>
<td>0.94±0.11</td>
</tr>
<tr>
<td></td>
<td>rSOD (n=10)</td>
<td>0.74±0.05</td>
</tr>
<tr>
<td>Epicardial</td>
<td>Control (n=9)</td>
<td>0.69±0.06</td>
</tr>
<tr>
<td></td>
<td>No lidocaine (n=6)</td>
<td>0.75±0.06</td>
</tr>
<tr>
<td></td>
<td>SOD + CAT (n=8)</td>
<td>0.61±0.07</td>
</tr>
<tr>
<td></td>
<td>rSOD (n=10)</td>
<td>0.53±0.04</td>
</tr>
</tbody>
</table>

**Creatine Kinase**

The curves for appearance of total creatine kinase in blood were similar for all groups (Figure 8). All three curves showed abrupt increases in creatine

<table>
<thead>
<tr>
<th></th>
<th>Nonischemic zone</th>
<th>Ischemic zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Coronary artery occlusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 min</td>
</tr>
<tr>
<td>Endocardial</td>
<td>Control (n=9)</td>
<td>1.05±0.11</td>
</tr>
<tr>
<td></td>
<td>No lidocaine (n=6)</td>
<td>1.11±0.14</td>
</tr>
<tr>
<td></td>
<td>SOD + CAT (n=8)</td>
<td>0.94±0.11</td>
</tr>
<tr>
<td></td>
<td>rSOD (n=10)</td>
<td>0.74±0.05</td>
</tr>
<tr>
<td>Epicardial</td>
<td>Control (n=9)</td>
<td>0.69±0.06</td>
</tr>
<tr>
<td></td>
<td>No lidocaine (n=6)</td>
<td>0.75±0.06</td>
</tr>
<tr>
<td></td>
<td>SOD + CAT (n=8)</td>
<td>0.61±0.07</td>
</tr>
<tr>
<td></td>
<td>rSOD (n=10)</td>
<td>0.53±0.04</td>
</tr>
</tbody>
</table>

SOD + CAT, superoxide dismutase and catalase; rSOD, recombinant superoxide dismutase.

*Significantly different from no lidocaine group, p < 0.05. No other significant differences were observed among the groups.
Figure 6. Line graphs of measurements of epicardial (EPI) (top panel) and endocardial (ENDO) (bottom panel) blood flows in the ischemic zone (ml/min) are summarized in the combined control group (n=15), dogs treated with superoxide dismutase and catalase (SOD+CAT, n=8), and dogs treated with recombinant superoxide dismutase (R-SOD, n=10). There were no differences in response to coronary artery occlusion (CAO) or reperfusion (CAR) among the treated groups and the combined control group.

kinase after reperfusion, reaching a peak at 2–5 hours after coronary artery occlusion. There was no significant difference in the level of creatine kinase in the rSOD group when compared with the untreated group. However, the level of creatine kinase appearing in the blood was significantly greater in the SOD and CAT–treated group at the time points noted in Figure 8.

Discussion

The results of the present investigation demonstrated a transient beneficial effect of rSOD treatment on reperfusion arrhythmias when administered before and immediately after coronary artery reperfusion after 90 minutes of coronary artery occlusion, but the results failed to demonstrate salvage of ischemic myocardium or ischemic myocardial function 1 week later.

The extent to which the effects of coronary artery occlusion and coronary artery reperfusion can be modified by treatment with oxygen free radical scavengers remains controversial. Several studies, which have been reviewed recently, have demonstrated favorable effects in reducing reperfusion arrhythmias by treatment with oxygen free radicals as was observed in the present investigation. The ultimate effect of treatment with SOD or CAT on infarct size is more controversial. The majority of studies demonstrating markedly beneficial effects of treatment with oxygen free radical scavengers have been conducted in acutely prepared anesthetized dogs, while the use of recombinant superoxide dismutase (R-SOD) and CAT in these studies was noted in Figure 6.

Figure 7. Line graph of effects of 90 minutes of coronary artery occlusion (CAO) followed by reperfusion (CAR) on arrhythmia beats per minute are shown for the nine dogs in the control group receiving lidocaine, eight dogs treated with superoxide dismutase plus catalase (SOD+CAT), and 10 dogs treated with recombinant superoxide dismutase (R-SOD). The time of treatment is indicated by the solid black bar. Dogs treated with R-SOD but not SOD+CAT exhibited fewer arrhythmic beats per minute during CAR. This was statistically significant (*) at 2 hours after CAR.

Figure 8. Line graph of effects of coronary artery occlusion (CAO) and reperfusion (CAR) on measurements of plasma creatine kinase (CK) level (IU/l) in the combined control group of 15 dogs, eight dogs treated with superoxide dismutase and catalase (SOD+CAT), and 10 dogs treated with recombinant superoxide dismutase (R-SOD). CK level peaked later and was significantly greater (*) at 7, 8, 10, 12, 14, 16, and 24 hours after CAO in the SOD+CAT group compared with the combined control group while the time to peak CK and rise in total CK were similar between the R-SOD–treated group and the combined control group.
preparations. Because the present investigation was conducted in conscious dogs, it is tempting to speculate that anesthesia and recent surgery stimulate the generation of oxygen free radicals, thereby making treatment more favorable in that setting. A study by Gallagher and colleagues, also conducted in conscious dogs, would tend to support this hypothesis. However, recent studies by Reimer and Jennings and coworkers, conducted in anesthetized dogs with an open chest, failed to demonstrate a beneficial effect of oxygen free radical scavengers or xanthine oxidase inhibitors. Thus, it is unlikely that simply the presence or absence of anesthesia and recent surgery is the only answer to the controversy. However, under the conditions of anesthesia or recent surgery, enhanced sympathetic tone and elevated catecholamine levels should result in generation of oxygen free radicals.

The commercially available SOD and CAT were contaminated by endotoxin, which can induce hypotension and increase body temperature, and this could explain the failure to demonstrate a beneficial effect. A decrease in arterial pressure, which reduces collateral blood flow, and an increase in body temperature, which augments metabolism, could increase the extent of infarction. On the other hand, endotoxin can cause a decrease in granulocytes, which should have the opposite effect. Although mean arterial pressure tended to fall in the SOD and CAT group, which would be consistent with endotoxemia, it should be noted that mean arterial pressure also tended to fall in the animals treated with rSOD, which was not contaminated with endotoxin. Furthermore, the decline in arterial pressure was relatively trivial. Nevertheless, to eliminate this complicating factor, we examined a group of dogs treated with rSOD that was demonstrated to be endotoxin free. In this group, a transient beneficial effect on arrhythmias was observed, but recovery of regional function and infarct size when measured 1 week after coronary artery reperfusion was not affected favorably. However, it is conceivable that SOD and CAT contaminated with endotoxin may have affected some of the results in prior studies.

The transient nature of the beneficial effects of rSOD suggests that oxygen free radicals may not simply occur in a rapid burst immediately after reperfusion but may be generated for longer periods of time during evolution of the infarct. In this connection, it is known that white blood cells, a major source of oxygen free radicals, also accumulate in the developing infarct as a later event. A recent preliminary report by Simpson et al supports this concept, in that prolonged treatment with Iloprost was more beneficial than treatment for only 2 hours after reperfusion. It is conceivable that an important difference between the present investigation and prior studies is the time after coronary artery reperfusion at which the final measurements were made. The majority of the studies demonstrating beneficial effects have been conducted in open chest anesthetized animals studied for a relatively short period of time (i.e., 2 days or less). Perhaps, in the present study, if treatment had been continued for a longer period or if animals had been studied for a shorter period of time, the beneficial effects of SOD would have been more evident. However, if this therapy is to be useful in patients, it must exert a beneficial effect for a far greater period of time than 1 week to be clinically efficacious.

One potential criticism of prior studies that did not find a beneficial effect was the failure to achieve sufficient blood levels of enzyme. Blood levels of SOD were measured in the present investigation and were found to be at least as high as the levels found in prior studies in open-chest anesthetized dogs that had favorable results.

Although it is not clear if arrhythmias were treated with lidocaine in all prior studies, they were treated in the present study. Previous studies demonstrated that lidocaine may reduce leukocyte migration of the ischemic area or impair the formation of oxygen free radicals. If these actions of lidocaine occurred in the present investigation, the beneficial effects of SOD should have been enhanced. This was not observed, and it is most likely that lidocaine administered in the present study did not affect the results. However, to be certain, a subgroup of control dogs was studied without administration of any lidocaine. Infarct size, expressed as a function of the area at risk, was not different in this subgroup when compared with the control subgroup that received lidocaine. A recent study also failed to demonstrate a beneficial effect of this drug on infarct size.

Ambrosio et al suggested that SOD was most effective in animals with large ischemic zones. To assess this possibility, all infarcts, expressed as a function of the area at risk, were compared for transmural collateral blood flows with a ratio of less than 0.10 (lower collateral blood flow) or with a ratio of more than 0.10 (higher collateral blood flow). Both analyses failed to demonstrate a preferential effect for SOD treatment, indicating that in the conscious dog the extent of collateral flow did not affect the response to SOD treatment.

Perhaps the most important determinant of infarct size in the dog is the amount of collateral flow available. It is for this reason that infarct size should be expressed not only as a function of area at risk, but also as a function of the available collateral blood flow. This type of analysis in the present investigation favored the group treated with rSOD, but no significant effects could be demonstrated. However, with longer periods of treatment with SOD after coronary artery reperfusion, a more favorable outcome could result. One final point, that is, the wide variation in availability of collateral blood flow and the inability to predict when collateral channels will open after coronary artery occlusion make the canine model particularly difficult when attempting to arrive at definitive
conclusions about the potential usefulness of any intervention affecting the ischemic myocardium.

Acknowledgments
We thank Diane Wathen for her help in preparing the tissue sample for pathology, Dr. Ronald Callahan for conducting the Limulus test, and Juliet Washburn for typing the manuscript. We also thank the Chiron and Pharmacia Corporations for supplying the human recombinant superoxide dismutase and for assaying the plasma levels of this drug.

References
2. Reimer KA, Jennings RB: The “wavefront phenomenon” of myocardial ischemic cell death: II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. Lab Invest 1979;40:633–644
35. Richard RJ, Murry CE, Jennings RB, Reimer KA: Therapy to reduce free radicals during early reperfusion does not limit the size of myocardial infarcts caused by 90 minutes of ischemia in dogs. Circulation 1986;78:473–480
36. Urayzoe A, Reimer KA, Murry CE, Jennings RB: Failure of superoxide dismutase to limit size of myocardial infarction after 40 minutes of ischemia and 4 days of reperfusion in dogs. Circulation 1987;75:1237–1248
37. 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
42. Hess ML, Manson NH: Molecular oxygen: Friend and Foe: The role of the oxygen free radical system in the calcium paradox, the oxygen paradox and ischemia/reperfusion injury. *J Mol Cell Cardiol* 1984;16:969–985

**Key Words:** myocardial ischemia • oxygen free radicals • myocardial infarction • left ventricular wall thickness
Superoxide dismutase reduces reperfusion arrhythmias but fails to salvage regional function or myocardium at risk in conscious dogs.
J Nejima, D R Knight, J T Fallon, N Uemura, W T Manders, D R Canfield, M V Cohen and S F Vatner

Circulation. 1989;79:143-153
doi: 10.1161/01.CIR.79.1.143

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/79/1/143

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation 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 is online at:
http://circ.ahajournals.org/subscriptions/