Evidence for a reversible oxygen radical–mediated component of reperfusion injury: reduction by recombinant human superoxide dismutase administered at the time of reflow

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ABSTRACT  It has been suggested that the beneficial effects of reperfusing ischemic myocardium might be in part reversed by the occurrence of “reperfusion injury.” One possible mechanism could be the generation of oxygen free radicals. Superoxide dismutase enzymatically scavenges superoxide radicals by dismutation to hydrogen peroxide. This study tested the hypothesis that administration of recombinant human superoxide dismutase (h-SOD) at the time of reflow after a period of prolonged global ischemia would result in improved recovery of myocardial metabolism and function by preventing or reducing a potentially harmful component of reperfusion. We also sought to determine whether catalase, an enzymatic scavenger of hydrogen peroxide, was a necessary addition for optimal benefit. Langendorff perfused rabbit hearts were subjected to 30 min of normothermic (37°C) total global ischemia. At the moment of reperfusion, 12 control hearts received a 10 ml bolus of normal perfusate followed by 15 min of reperfusion with normal perfusate (group I), 12 hearts received 60,000 IU of h-SOD as a bolus followed by a continuous infusion of 100 IU/ml for 15 min (group II), and 12 hearts received 60,000 IU of h-SOD and 60,000 IU of catalase as a bolus followed by 100 IU/ml of both enzymes for 15 min (group III). Myocardial ATP and phosphocreatine (PCr) content and intracellular pH during ischemia and reperfusion were continuously monitored with 31P nuclear magnetic resonance (NMR) spectroscopy. During 30 min of normothermic global ischemia intracellular pH dropped from 7.11–7.18 to 5.58–5.80 in all three groups of hearts. Likewise myocardial PCr content fell rapidly to 7% to 8% and ATP fell more slowly to 29% to 36% of preischemic control content. After 45 min of reperfusion PCr recovered to 65 ± 5% of control in untreated (group I) hearts compared with 89 ± 8% in h-SOD–treated (group II) hearts (p < .01 vs group I) and with 83 ± 6% of control in h-SOD/catalase–treated (group III) hearts (p < .05 vs group I). Recovery of isovolumic left ventricular developed pressure was 68 ± 5% of control in h-SOD–treated (group II) hearts and 66 ± 6% of control in h-SOD/catalase–treated (group III) hearts after 45 min of reflow, compared with 48 ± 6% of control in untreated (group I) hearts (p < .005 for groups II and III vs group I). The NMR data confirmed equal depletion of ATP and PCr content in all three groups of hearts. Thus, despite an equally severe ischemic insult, hearts receiving h-SOD at the time of reperfusion recovered significantly better PCr content and left ventricular function. Catalase provided no additional benefit. These results clearly demonstrate that reperfusion of ischemic myocardium results in a separable component of myocardial injury, which can be reduced or prevented by administration of the oxygen free radical scavenger, h-SOD, at the time of reperfusion.

oxide anions (\(\cdot O_2\)), at the time of reoxygenation. We have recently documented, using electron paramagnetic resonance techniques, a marked increase in free radical concentration within the first 30 sec of post-ischemic reperfusion in isolated rabbit hearts.\(^5\)

This study was designed to test two hypotheses. The first hypothesis was that the administration of the oxygen free radical scavenger, recombinant human superoxide dismutase (h-SOD), beginning at the time of ischemia, would result in improved recovery of myocardial high-energy phosphates and left ventricular function by preventing or reducing a potentially harmful component of cell damage caused by reperfusion. Since hydrogen peroxide is the product of the dismutation reaction catalyzed by h-SOD and since hydrogen peroxide itself may be potentially harmful,\(^6\) catalase would be a logical addition to h-SOD. Therefore, the second hypothesis was that catalase, a hydrogen peroxide scavenger, would provide improved metabolic and/or functional recovery compared with that obtained with h-SOD alone.

Materials and methods

Isolated heart preparation. Female New Zealand white rabbits (1.5 to 2.0 kg) were heparinized and anesthetized, and their hearts were removed and quickly placed into cold perfusate. The ascending aorta was cannulated and the hearts were perfused retrogradely under constant pressure (80 mm Hg) with a modified Krebs-Ringer’s bicarbonate (KRB) buffer solution containing 117 mM sodium chloride, 6.0 mM potassium chloride, 3.0 mM calcium chloride, 1.0 mM magnesium sulfate, 0.5 mM EDTA, 16.7 mM glucose, and 24 mM sodium bicarbonate, pH 7.4. The perfusate was bubbled continuously with a gas mixture containing 95% oxygen and 5% carbon dioxide. The hearts were paced at 175 beats/min with a wick electrode containing saturated potassium chloride, encased in polyethylene tubing, placed at the apex of the right ventricular cavity and connected to a Grass SD-9 stimulator. To assess contractile function, a latex balloon was inserted into the left ventricular cavity through the mitral opening and secured with a ligature that included the left atrial remnants. The balloon was tied to the end of a 100 cm segment of PE 190 tubing, carefully purged of air bubbles and connected via a three-way stopcock to a Statham P23Db transducer. Left ventricular pressure was recorded with a Brush two-channel direct-writing recorder. The balloon was initially inflated via a syringe with a volume of saline sufficient to produce an end-diastolic pressure of 10 mm Hg. All subsequent measurements of left ventricular developed pressure, calculated as the difference between peak systolic and end-diastolic pressure, were made at this same end-diastolic volume (i.e., isovolumic). Coronary flow was measured intermittently by vacuum aspiration of perfusate overflow.

Nuclear magnetic resonance (NMR) methods. \(^3\)P-NMR spectra were obtained with a Bruker WH 180 spectrometer at 4.23 Tesla. At this field strength phosphorus resonates at 72.89 MHz. The diameter of the probe was 25 mm. The instrument was operated in the pulsed Fourier transform mode and interfaced to a Nicolet 1280 computer and the data were collected on high-density magnetic disks. Because of the field stability of the superconducting magnet, field/frequency lock was not required. Five minute proton decoupled spectra were collected from transients following 45 degree pulses delivered at 2 sec intervals, conditions previously documented to result in minimal spectral saturation.\(^7\) The data were accumulated with a 2K table at a 3000 Hz spectral width.

Estimation of tissue intracellular pH from NMR spectra. Measurement of intracellular pH was determined from the chemical shift of the inorganic phosphate peak by the following equation:

\[ p\text{H}_i = \frac{pK - \log \left( \frac{\delta_S - \delta_B}{\delta_A - \delta_B} \right)}{\delta_A - \delta_S} \]

To minimize tissue inhomogeneity effects, chemical shift values were measured relative to the resonance of phosphocreatine (PCR), whose peak position is relatively pH independent over the range of pH values encountered in this study (pK_\text{A} = 4.6). The constants used in this equation are pK = 6.90, \(\delta_A = 3.290\) ppm, and \(\delta_B = 5.805\) ppm, as previously reported.\(^8\)

Quantitation of metabolites from NMR spectra. Estimations of tissue PCR and ATP content were obtained by integration of the areas under the individual peaks. A Hewlett-Packard digitizer was used to perform the area integrations. Quantitative data thus derived for PCR and ATP are expressed as a percentage of the preischemic control content.

Experimental protocol. After a 10 min equilibration period, two 5-min preischemic control NMR spectra were obtained and control left ventricular developed pressure and end-diastolic pressure were recorded. Total interruption of aortic inflow was accomplished by cross-clamping the perfusion line. During 30 min of global ischemia, hearts were maintained at 37° C by a continuous nonrecirculating flow of warm oxygenated (95% \(O_2\), 5% \(CO_2\)) perfusate around the heart. At the onset of ischemia, the balloon was deflated and the pacemaker turned off. Forty-five minutes of normothermic reperfusion followed the release of the clamp. Ventricular pacing was reinstalled at the onset of reflow. The intraventricular balloon was reinflated 15 min after initiating reflow, just before the first measurement of function, with the same volume removed at the onset of ischemia. Recovery of left ventricular developed pressure was expressed as a percentage of the preischemic control value. Coronary flow was measured before ischemia and after 15, 30, and 45 min of reperfusion. A side arm in the perfusion line attached just above the heart allowed administration of a bolus of either a solution containing free radical scavenger or of standard KRB perfusate at the time of reflow. Serial 5 min NMR spectra were collected throughout the ischemic and reperfusion periods.

Thirty six hearts were divided into three experimental groups:

**Group I.** Twelve hearts received a 10 ml bolus of standard KRB perfusate at the time of reflow followed by 45 min of normothermic reperfusion with standard perfusate.

**Group II.** Twelve hearts received 60,000 U of h-SOD (specific activity 3200 IU/mg) as a 10 ml bolus at the time of reflow, followed by a continuous infusion of 100 IU/ml during the first 15 min of reflow; h-SOD was dissolved in warm (37° C) KRB perfusate. h-SOD was expressed in *Escherichia coli* and supplied courtesy of Bio-Technology General Corp., New York, NY. This enzyme differs from the naturally occurring human red blood cell SOD only in that its N-terminal is not acetylated. The enzyme is physiologically and pharmacologically indistinguishable from the natural enzyme (data on file BTG Corp., IND application to FDA approved).

**Group III.** Twelve hearts received a 10 ml bolus of perfusate containing 60,000 IU of h-SOD and 60,000 IU of bovine liver catalase (thymol free, Sigma Chemical Co., St. Louis, specific activity 11,000 IU/mg), followed by 15 min of normothermic reperfusion with 100 IU/ml of each enzyme.
After the initial 15 min of intervention, group II and group III hearts were switched to standard KRB perfusate for the remaining 30 min of the 45 min normothermic reperfusion period.

Statistical analysis. Data are expressed as mean ± SEM. Statistical analysis employed two-way analysis of variance (ANOVA) with repeated measures comparing groups I vs II, groups I vs III, and groups II vs III, respectively. When the overall ANOVA revealed statistical significance of $p < 0.05$ or greater, differences at individual time points were tested by repeated unpaired t tests. Because multiple comparisons were involved, a Bonferroni correction was used. Since three comparisons were made, the conventional $p$ value of 0.05 was divided by three to give $p \leq 0.0167$ as the minimum acceptable level of significance. When $p \leq 0.05$ is given in text for an individual comparison, this indicates a corrected $p$ value of $\leq 0.0167$.

Results

Quantity of free radical scavengers administered. Since after the initial 60,000 IU bolus, hearts were perfused with a known concentration of h-SOD or h-SOD plus catalase during the first 15 min of reperfusion, the total quantity of enzymes delivered to each heart varied with coronary flow. The total dose of h-SOD received by group II hearts ranged from 94,000 to 106,000 IU, i.e., 100,000 IU ± 6%. A similar range of ±6% for the total dose was also found for group III hearts, which received a fixed concentration of both h-SOD and catalase during the first 15 min of reperfusion.

Metabolic variables during global ischemia and reperfusion

Time course of intracellular pH. Thirty minutes of normothermic total global ischemia induced severe myocardial acidosis, as evidenced by a progressive fall in intracellular pH (pHi) from initial baseline values of 7.11–7.18 to 5.58–5.80 at the end of the ischemic period, with a similar time course for all three groups of hearts (figure 1). After reperfusion, pHi rose rapidly in all three groups. Within the first 15 min of reflow, pHi reached normal levels of 7.12 ± 0.04 in group II (h-SOD treated) and 7.14 ± 0.02 in group III (h-SOD/catalase treated) hearts. In contrast, group I (control) hearts reached a pHi of only 6.95 ± 0.04 during the initial 15 min of reflow (p < 0.05 for groups II and III vs group I). Thereafter, pHi in group I hearts rose steadily, approaching pHi in group II and III hearts near the end of the 45 min reperfusion period (p < 0.05 for groups II and III vs group I by two-way ANOVA).

Time course of myocardial PCr content. Before the onset of ischemia, control spectra revealed PCr/ATP ratios of 1.53 ± 0.19, 1.51 ± 0.28, and 1.64 ± 0.27 for groups I, II, and III, respectively (p = NS). Myocardial PCr content decreased rapidly during the early minutes of global ischemia, falling to 12% to 16% of

![Figure 1](http://circ.ahajournals.org/)
control content within 10 min and reaching a minimum of 7% to 8% of control after 30 min of ischemia in all three groups (figure 2). PCr content recovered rapidly after reperfusion. In all three groups of hearts, PCr content peaked 15 min after reflow. In group I hearts PCr demonstrated recovery of 60 ± 9% of the preischemic control value within 5 min, 72 ± 6% after 15 min, and 65 ± 5% at the end of 45 min of reflow. Both groups of treated hearts recovered significantly greater PCr content than control (group I) hearts. Group II hearts, which received h-SOD alone, recovered 107 ± 10% of control PCr content 15 min after reflow, thus exhibiting a small "overshoot" above preischemic control values. PCr content then fell gradually to 89 ± 8% during the remainder of the 45 min reflow period, during which time the intraventricular balloon was inflated (p < .01 vs group I by ANOVA). Finally, h-SOD/catalase–treated hearts (group III) demonstrated recovery of PCr content of 91 ± 7% after 15 min of reperfusion and 83 ± 6% after 45 min of reflow (p < .05 vs group I and p = NS vs group II by ANOVA). Recovery of PCr content in group II hearts was significantly different from control (group I) hearts at all but one point during the reperfusion period (figure 2) (p < .05 by Bonferroni corrected t tests). In addition, the slope of the PCr content vs time during the last 30 min of reperfusion for group II hearts was significantly different from group I and III hearts (p < .05 for the PCr vs time interaction). There were no significant differences in recovery of PCr content between groups II and III hearts by ANOVA.

Time course of myocardial ATP content. Myocardial ATP content decreased progressively during 30 min of normothermic global ischemia, reaching a minimum of 29% to 34% of control content by the end of the ischemic period in all three groups of hearts (p = NS) (figure 3). During the first 15 min of reperfusion there was initially comparable recovery of ATP content in all three groups of hearts to 43% to 47% of control. Reinfusion of the intraventricular balloon 15 min after the onset of reflow would be expected to increase myocardial energy consumption (demand). From this point on, ATP content declined slightly to 40 ± 3% in the group I hearts. In contrast, ATP content rose slightly in the group II hearts to 49 ± 3% of control after 45 min of reperfusion (p < .05 vs group I by ANOVA). ATP content in group III hearts remained relatively constant throughout reperfusion, 43 ± 4% of control at the end of the reperfusion period (p = NS vs groups I and II by ANOVA). Although significantly different by ANOVA, the small differences in recovery of ATP content for group II vs group I were not significantly different by individual t tests with the Bonferroni correction.

**FIGURE 2.** Time course of myocardial PCr content, expressed as a percentage of the preischemic control value (% control), during 30 min of normothermic ischemia and 45 min of reperfusion. Format as in figure 1. Two-way ANOVA showed that recovery of PCr during reperfusion is significantly different in group II vs group I (p < .01) and in group III vs group I (p < .05). The difference between groups II and III was not significant.
Recovery of left ventricular function after reperfusion

Left ventricular developed pressure. Preischemic baseline values of left ventricular developed pressure did not differ significantly among the three groups of hearts (106 ± 6 mm Hg in group I, 102 ± 5 mm Hg in group II, and 111 ± 4 mm Hg in group III). After 15 min of reperfusion group I hearts recovered 32 ± 4% of control developed pressure, gradually rising to 48 ± 4% of control after 45 min of reflow (figure 4). In contrast, group II hearts recovered significantly better left ventricular function than group I hearts (p < .005 by ANOVA). Group II hearts recovered 50 ± 5% of preischemic control developed pressure after 15 min of reperfusion (p < .05 vs group I by corrected t test) and further recovered to 68 ± 5% of control after 45 min of reperfusion (p < .01 vs group I by corrected t test). Recovery of developed pressure in group III hearts was virtually identical to that of group II hearts treated with h-SOD alone (p < .005 versus group I and p = NS vs group II by ANOVA, and p < .05 at each time point by corrected t tests).

End-diastolic pressure. Baseline end-diastolic pressure was 9.8 ± 0.5 mm Hg in group I, 9.7 ± 0.5 mm Hg in group II and 9.6 ± 0.5 mm Hg in group III hearts (p = NS). After 15 min of reperfusion, reinfalation of the intraventricular balloon with the same volume of fluid that had been withdrawn at the onset of the ischemic period, resulted in a higher left ventricular end-diastolic pressure in all three groups of hearts. End-diastolic pressure then declined slowly during the remainder of the reperfusion period (figure 5). In both groups II and III end-diastolic pressure was significantly lower than that in group I hearts throughout the reperfusion period (p < .005 by ANOVA) (figure 5). At the end of 45 min of reflow, end-diastolic pressure was 47 ± 5 mm Hg in group I hearts compared with 31 ± 4 and 30 ± 5 mm Hg in group II and group III hearts, respectively.

Coronary flow after reperfusion. Coronary flow measured before the onset of ischemia was 35 ± 3 ml/min in group I, 39 ± 2 ml/min in group II, and 35 ± 2 ml/min in group III hearts (p = NS). Throughout the 45 min of reperfusion, coronary flow was significantly higher in groups II and III (treated) than in group I (untreated) hearts (p < .01 vs controls by ANOVA; figure 6). At the end of the reperfusion period, coronary flow in untreated (group I) hearts was 59 ± 5% of control compared with 72 ± 5% and 69 ± 7% of control in groups II and III (treated hearts), respectively (p = NS for groups II and III vs group I by corrected t tests).

Discussion

Our results demonstrate that administration of the oxygen free radical scavenger, h-SOD, at the time of reperfusion results in improved recovery of both PCr content and left ventricular function in isolated per-
fused rabbit hearts subjected to global ischemia. These findings also support the hypothesis that reoxygenation is a major factor in the genesis of reperfusion injury, thereby adding to the myocardial damage caused by ischemia itself.

Oxygen radicals, which are characterized by the presence of an unpaired electron, can either initiate a chain reaction leading to generation of other free radicals or directly attack unsaturated fatty acids within cell membranes. Peroxidation of membrane lipids has been shown to result in increased membrane permeability, decreased calcium transport into the sarco-

**FIGURE 4.** Recovery of left ventricular developed pressure (DP), expressed as percent control after 30 min of normothermic ischemia and 15, 30, and 45 min of reperfusion. Format as in figure 1. Both groups II and III were statistically different from group I (p < .005 by ANOVA) (*p < .05 vs group I; **p < .01 vs group I by Bonferroni corrected t tests).

**FIGURE 5.** Recovery of left ventricular end-diastolic pressure (EDP) after 30 min of normothermic ischemia and 15, 30, and 45 min of reperfusion. Format as in figure 1. Both groups II and III were statistically different from group I (p < .005 by ANOVA). *p < .05 by Bonferroni corrected t tests.
plasmic reticulum,\textsuperscript{15} and altered mitochondrial function.\textsuperscript{16, 17} Myocardial cells are constantly exposed to small quantities of superoxide anions, which are produced in mitochondria during electron transport, by xanthine oxidase–catalyzed reactions in vascular endothelium, during prostaglandin synthesis in the cytoplasm, and by activated neutrophils in the extracellular space. Myocardial cells possess an enzyme, SOD, that catalyzes the dismutation of superoxide anions to hydrogen peroxide and oxygen by the equation \( \cdot O_2^- + \cdot O_2^- + 2H^+ \rightarrow H_2O_2 + O_2 \textsuperscript{18} \). Metabolism of H\textsubscript{2}O\textsubscript{2} to water and oxygen is then accomplished either by catalase or glutathione peroxidase. Acute myocardial ischemia profoundly alters this balance, by decreasing the cellular levels of SOD,\textsuperscript{10} while at the same time promoting the accumulation of metabolic end products such as hypoxanthine and xanthine, which would set the stage for a subsequent “burst” of free radical generation at the time of reoxygenation.\textsuperscript{5} Under these conditions the ability of the endogenous enzyme system to scavenge superoxide anions may be exceeded, resulting in the accumulation of highly reactive oxygen radicals.

Several biochemical pathways could be responsible for the generation of oxygen free radicals at the time of reperfusion of ischemic myocardium. Hypoxanthine and xanthine have been shown to accumulate during myocardial ischemia, as ATP is stepwise degraded.\textsuperscript{19} Metabolism of hypoxanthine and xanthine is normally catalyzed by xanthine dehydrogenase. However, during ischemia, xanthine dehydrogenase is converted to xanthine oxidase.\textsuperscript{20} Conversion of hypoxanthine to xanthine and xanthine to uric acid, when catalyzed by the oxidase form of the enzyme, is associated with the generation of superoxide anions.\textsuperscript{20} Other possible mechanisms of free radical formation include certain steps in prostaglandin synthesis,\textsuperscript{21, 22} oxidation of tissue catecholamines,\textsuperscript{23} and release of superoxide anions by phagocytically active neutrophils migrating into ischemically damaged tissue.\textsuperscript{24} This latter mechanism, however, would not explain generation of free radicals within the first several minutes of reperfusion nor the benefits observed in the present study, which used a non–blood containing perfusate.

Previous studies from this laboratory have demonstrated the usefulness of \(^{31}\text{P}-\text{NMR}\) spectroscopy for the biochemical evaluation of interventions designed to reduce ischemic damage.\textsuperscript{7, 25} \(^{31}\text{P}-\text{NMR}\) spectroscopy allowed us to document that the severity of the ischemic insult was identical in each of the experimental groups before the administration of the intervention specifically designed to reduce or prevent reperfusion injury.

The \(^{31}\text{P}-\text{NMR}\) technique also allowed continuous and noninvasive assessment of subsequent recovery of myocardial metabolism. In the present study, PCr content fell progressively to less than 10% of control after 30 min of ischemia in all groups of hearts. Reperfusion

\textbf{FIGURE 6.} Coronary flow, expressed as a percentage of the preischemic control value, after 30 min of normothermic ischemia and 15, 30, and 45 min of reperfusion. For both groups II and III coronary flow for the overall reperfusion period was significantly higher than for group I (control hearts) (\( p < .01 \) by ANOVA).
promptly restored PCr content to near-normal levels in both groups of treated hearts, whereas PCr recovery in untreated hearts remained below 70% of preischemic control throughout the reperfusion period. As can be seen in figure 2, these differences in PCr content were already evident in the 31P-NMR spectra obtained within the first 5 min of reperfusion. Several possible mechanisms could be responsible for this impaired recovery of PCr content during the early minutes of postischemic reperfusion. Recent studies have demonstrated that mitochondrial function can be damaged by exposure in vitro to exogenous free radicals17 or to high concentrations of oxygen, which presumably increases endogenous production of oxygen radicals.26 It is noteworthy that intracellular pH remained significantly lower in untreated than in treated hearts during the first 15 min of reperfusion. Since the equilibrium constant for the creatine kinase reaction is extremely sensitive to pH,27 even a small reduction in pH could slow the rate of recovery of PCr content. Finally, it is possible that significant leakage of creatine and/or phosphate occurred from more severely injured cells. Depletion of these substrates would also impair recovery of PCr content after reperfusion.

The small differences in recovery of ATP content between treated and untreated hearts are in marked contrast to the larger differences noted for PCr content and ventricular function. After 15 min of reflow ATP contents were virtually identical in all groups of hearts, a time when PCr contents were already markedly different. Differences in ATP content remained small among groups of hearts even at the end of the 45 min reperfusion period. This weak correlation between recovery of ATP content and contractile function in postischemic hearts has also been reported by other investigators.28-30 We might speculate that higher rates of ATP synthesis in better protected hearts are in part balanced by increased rates of ATP utilization by hearts demonstrating better ventricular function. In addition, loss of ATP precursors during postischemic reperfusion, would also tend to limit regeneration of ATP content.31 If operative, one or both of these mechanisms would tend to minimize differences in recovery of ATP content.

Differences in end diastolic pressure after reperfusion between treated and untreated hearts could be explained by greater ventricular stiffness or "diastolic contracture," resulting from increased uptake of water and calcium ions through damaged sarcolemmal membranes during reperfusion. Increases in myocardial edema and diastolic contracture have been shown to result in a parallel leftward shift of the end diastolic pressure-volume relationship and reduction in the unstressed volume of the left ventricular cavity.32 The lower coronary flow noted during reperfusion in untreated hearts could also be the result of greater cellular uptake of sodium and water and thereby greater extracellular compression of capillaries (i.e., the "no-reflow phenomenon").33,34 Microvascular injury might be expected to be proportional to the degree of overall myocardial damage. Furthermore, since xanthine oxidase has been shown to be in highest concentration in vascular endothelium,35 generation of superoxide anions within endothelial cells would act to accentuate microvascular injury.

That treatment with free radical scavengers reduces infarct size after regional myocardial ischemia and reperfusion in a canine preparation has previously been reported by Jolly et al.36 and Werns et al.37 These results provide strong support for the hypothesis that oxygen radical formation is a major contributor to reperfusion injury. In these studies, free radical scavengers were administered beginning either 15 min before the end of the ischemic period,37 or before coronary occlusion, as a pretreatment.36,37 However, this approach does not rule out the possibility that the intervention had an effect during the critical later minutes of the ischemic period. By our study design, administration of h-SOD was begun with a bolus at the time of reflow and thus was not present during the period of ischemia. Therefore, the improved recovery of myocardial function and PCr content observed in the present study must be the result of an oxygen radical-mediated component of cell damage, which occurs at the time of and as a consequence of postischemic reperfusion. With the 31P-NMR data confirming the presence of an equivalent ischemic insult, our results support the previous literature and provide strong evidence for the existence of a separable component of myocardial injury that can be related to reflow rather than to ischemia itself.

Dismutation of superoxide radicals by SOD results in the formation of hydrogen peroxide. Hydrogen peroxide has been shown in several experimental preparations to be cytotoxic, either by a direct effect6 or through the superoxide-mediated generation of more reactive hydroxyl radicals (·OH) by the Haber-Weiss and Fenton reactions.38,39 Several studies, including one from our own institution, have demonstrated beneficial effects using the combination of SOD and the hydrogen peroxide scavenger, catalase, on the recovery of contractility30,40-43 as well as the extent of myocardial necrosis.36 Recently, Werns et al.37 found that SOD alone produced the same reduction in infarct size
that this same laboratory had previously reported with the combination of SOD plus catalase. 36 These results and the results of the present study would suggest that endogenous levels of catalase and/or glutathione peroxidase are sufficient to catabolize hydrogen peroxide as it is formed by the dismutation of superoxide radicals catalyzed by SOD. Alternatively, the quantity of SOD administered exogenously may have been sufficient to scavenge the superoxide radicals as fast as they were generated, thereby preventing the formation of hydroxyl radicals, by reactions that require the simultaneous presence of hydrogen peroxide and superoxide anions. 39, 44 This proposed mechanism might also help explain the divergent results found by Myers et al. 45 using catalase alone in a hypoxia/reoxygenation model. Since beneficial effects have also been reported with the hydroxyl ion scavenger mannitol, 46 taken together these results could suggest that it is the more reactive hydroxyl radical that must be avoided rather than its less reactive precursor, superoxide.

In conclusion, our results clearly demonstrate that reperfusion of ischemic myocardium results in a separable and potentially reversible component of myocardial damage, which can be reduced or prevented by administration of the oxygen free radical scavenger, h-SOD, at the time of reperfusion. Although we must be cautious extrapolating these results to the clinical setting, addition of h-SOD to a thrombolytic infusion might provide a means of reducing reperfusion injury in patients with acute myocardial infarction. This issue is becoming increasingly important as thrombolytic therapy has become routine treatment in many hospitals for patients presenting early after the onset of symptoms. 47

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