

Reduction in experimental infarct size by recombinant human superoxide dismutase: insights into the pathophysiology of reperfusion injury

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ABSTRACT To determine the importance of reperfusion injury and the ability of the free-radical scavenger recombinant human superoxide dismutase (h-SOD) to prevent it, open-chest dogs underwent 90 min of proximal circumflex coronary artery occlusion, and only at the moment of reperfusion received either h-SOD (400,000 IU bolus into the left atrium followed by a 300,000 IU iv infusion over 1 hr) or saline. After 48 hr the surviving animals were killed and measurements were made of the risk region (by postmortem angiography) and infarct size (by gross pathology). All measurements were made by investigators blinded to treatment given, and the code was broken only at the end of the study. Hemodynamic variables and collateral flow during ischemia were similar in the two groups. Infarct size in control animals ($n = 8$) averaged $22.4 \pm 3.1\%$ of the left ventricle and $52.2 \pm 7.1\%$ of the risk region, compared with $13.3 \pm 0.8\%$ of the left ventricle and $33.6 \pm 2.1\%$ of the risk region in h-SOD-treated dogs ($n = 8$) ($p < .05$). Infarcts in treated animals were not only smaller, but also exhibited a distinctive "patchiness," suggesting protection along vascular distributions. Furthermore, analysis of the relationship between infarct size and collateral flow measured during ischemia in the two groups indicated that protection by h-SOD was greatest in animals with the lowest collateral flows. This study supports the concept that reperfusion of ischemic myocardium results in a separate component of cell damage, presumably linked to the generation of oxygen free radicals on reflow. Since the h-SOD preventable reperfusion component of injury was most pronounced in hearts with the most severe ischemia, scavenging of oxygen radicals at the time of reflow may offer a novel and particularly promising therapeutic approach for the protection of ischemic myocardium.

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IT HAS BEEN convincingly demonstrated that timely reperfusion of ischemic myocardium can reduce the amount of necrosis after coronary artery occlusion.¹⁻⁵ There is also evidence to suggest that this beneficial effect might be blunted because reperfusion, while terminating ischemia, may also cause further damage

to jeopardized cells.^{6,7} This issue has become paramount with the recent introduction of clot-selective intravenous thrombolytic agents⁸ and the widespread use of coronary angioplasty.⁹ Reperfusion has become a practical option for an increasing number of patients early in the course of acute myocardial infarction.

Among several possible mechanisms, generation of oxygen free radicals at the time of reperfusion (reoxygenation) has been considered a major causative factor for myocardial reperfusion injury.¹⁰⁻¹³ In this regard, studies using electron paramagnetic resonance spectroscopy have demonstrated that oxygen free radicals are generated on reperfusion of ischemic hearts.^{14, 15}

Studies in a number of experimental preparations of ischemia and reperfusion have recently documented the beneficial effect on myocardial cell viability of superoxide dismutase (SOD), an enzymatic scavenger of oxygen radicals.^{12, 13, 16, 17} In these studies, SOD was administered alone^{16, 17} or in combination with cata-

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lase^{12, 13} beginning before the onset of ischemia^{12, 13, 16, 17} or 15 min before reperfusion.¹³ Although it seems likely that the major benefit of SOD was related to a decrease in reperfusion injury, the timing of enzyme administration was such that at least part of the benefit could have been related to a modification of the ischemic component of cell injury. In addition, all of these studies used SOD purified from bovine erythrocytes, rather than the human enzyme, which has recently become available through recombinant DNA technology.

In the present study we tested whether recombinant human SOD (h-SOD), administered at the moment of reflow after a period of coronary artery occlusion, would result in reduction of myocardial cell necrosis as compared with that after reperfusion alone. We also measured regional myocardial blood flow with the use of radioactive microspheres during ischemia and reflow to assess the relationship of the severity of ischemia and postischemic flow to any observed benefit of h-SOD with respect to reducing infarct size.

Methods

Surgical preparation. Twenty-nine mongrel dogs of both sexes (weight 20 to 25 kg) were anesthetized with sodium pentobarbital (30 mg/kg iv) and ventilated with room air. A left thoracotomy was performed aseptically and polyethylene catheters were advanced into the left atrium and into the thoracic aorta through the left internal mammary artery. The circumflex coronary artery was isolated proximally to any major marginal branch. Lead II of the electrocardiogram and aortic and left atrial pressures were continuously monitored on a direct-writing recorder.

Experimental protocol. After baseline measurements of hemodynamics and myocardial blood flow, the circumflex coronary artery was occluded by a snare. Coronary flow was measured again 5 min after occlusion and 5 min before reflow. At this time the animals were randomly assigned to either the treated or the control group. At the moment of reflow treated dogs received 400,000 IU of h-SOD (specific activity 3000 IU/mg) as a 10 ml bolus into the left atrium over 30 sec, followed by a continuous intravenous infusion of 300,000 IU over 60 min. The Cu-Zn form of human SOD was expressed in *Escherichia coli* and was obtained courtesy of Bio-Technology General Corp., New York. The enzymatic activity of the preparation was assayed according to the method of McCord and Fridovich.¹⁸ The enzyme was supplied as a lyophilized powder, endotoxin free, and was dissolved in sterile saline before use. Control dogs received an equivalent volume of saline given by the same routes. In both groups treatment was instituted simultaneously with the release of the coronary occlusion, after 90 min of ischemia. Ten minutes after reperfusion, myocardial blood flow was again assessed. The distal ends of the atrial and arterial lines were then externalized at the back of the chest; the thoracotomy was closed, air was evacuated from the chest, and penicillin (1 million units) and streptomycin (1 g) were given intramuscularly. One hour after reperfusion the infusion was stopped and the dogs were returned to their cages after recovery from anesthesia. Forty-eight hours later the animals were reanesthetized and killed by an overdose of KCl. Each heart was then removed and processed.

Delineation of the anatomic risk region. The size of the anatomic risk region, or occluded vascular bed, was measured by postmortem stereoscopic angiography, as previously described.¹⁹ Briefly, the coronary arteries were cannulated separately at their origins and injected simultaneously under controlled pressure (160 mm Hg) with a barium sulfate gelatin mass. Each heart was fixed in formalin and then sliced into four to six transverse sections in a plane parallel to the atrioventricular groove. Stereoscopic x-ray pictures were taken of the whole heart as well as of each slice. The boundaries of the risk region were marked on each radiographed section by an observer who had no knowledge of the treatment given. The arterial branches were identified as originating from a normal vessel or from the circumflex artery distal to the occlusion. The course of each branch was followed from ring to ring and a mark was placed where the terminal ramifications of an occluded artery interdigitated with those of a normal vessel. Good interobserver reproducibility has been shown previously for this technique.¹⁹

Measurement of infarct size. The heart slices were dissected free of the right ventricle, large epicardial vessels, and fat, and weighed. Color photographic transparencies were taken of each ring and projected onto a sheet of paper. The contour of each ventricular ring was then traced. Areas of infarction were identified visually and marked on each tracing by an observer who was unaware of the treatment given. The code of the group assignment was broken only at the end of the study. The outlines of the occluded bed (risk region) were transferred to the tracings from the corresponding radiographs after alignment by means of natural (papillary muscles and cavity shape) and metallic markers. Areas of the left ventricular rings, risk regions, and infarcts were measured by planimetry, and masses of infarct and risk region were calculated by multiplying the appropriate area ratios by the weight of each ring and summing the values for the whole heart.

Measurement of regional myocardial blood flow. Collateral flow to the occluded region was measured with 16 μ m diameter radioactive microspheres (NEN-TRAC, New England Nuclear, Boston). Measurements were made at baseline, 5 and 85 min after occlusion, and 10 min after the onset of reperfusion. For each flow measurement, 2 million spheres labeled with ¹⁴¹Ce, ¹¹³Sn, ¹⁰³Ru, ⁹⁵Nb, or ⁴⁶Sc were injected into the left atrium, followed by a 10 ml saline flush. Microspheres were obtained as 2 mCi of nuclide in 10 ml of 10% dextran. Microsphere vials were vigorously agitated on a mechanical mixer for 2 to 3 min before use. Starting just before injection and continuing for 2 min afterward, a reference arterial blood sample was withdrawn by a Harvard pump at a constant rate of 2.16 ml/min.

Sampling for regional myocardial blood flow was made transmurally in the center of the risk region and in the non-ischemic anterior wall. Samples (0.5 to 1.5 g) were divided into inner and outer halves, weighed, and counted for radioactivity with the reference blood samples in a scintillation counter (Packard 5986) at appropriate energy windows. Myocardial blood flow (ml/min/g) was calculated by standard methods.²⁰ Blood flows for each region were found by pooling appropriate myocardial samples. The preocclusion content of microspheres in each ischemic region expressed relative to that in the non-ischemic area was used to quantify the combined effects of microsphere loss, local edema, hemorrhage, and inflammatory cell infiltrate, and was used to correct flows for these factors.²⁰

Histologic study. To check the accuracy of the estimation of the infarct size by gross pathology, samples from each heart were cut from the left ventricular ring that corresponded to the middle of the risk region. The specimens, encompassing the ischemic area, were dehydrated and embedded in paraffin. Five micron thick sections were cut from the upper surface, stained with hematoxylin-eosin, and analyzed under low-power light

microscopy. Each microscopic slide was then projected onto a sheet of paper and the contours of the whole specimen and of the infarcted portion were drawn. Areas were then measured by planimetry as described above; the extent of necrosis, as determined histologically, was then compared with the value obtained by macroscopic examination of the same specimens.

Statistical analysis. Data are presented as the mean \pm SEM. Student's *t* test for unpaired data was used for statistical comparisons between groups. The correlation of collateral blood flow versus infarct size was assessed by linear regression analysis.

Results

Mortality. Six dogs died of ventricular fibrillation 10 to 20 min after the occlusion. The remaining dogs were randomly assigned to the treatment ($n = 12$) or the control group ($n = 11$). One dog in each group developed ventricular fibrillation within 1 hr after reperfusion. Both animals were successfully resuscitated. Five animals (two controls and three treated) died overnight, less than 24 hr from the occlusion, and were not included in the study. No animals died during the

remaining reperfusion period. Finally, one dog in each group was retrospectively excluded from the study because a significant reduction in blood flow was not achieved (i.e., flow 20% or less of baseline in the center of the ischemic region). Since the size of the anatomic risk region in these animals was comparable to that in the remaining dogs, the high flows during ischemia were considered to represent failures of occlusion.

Hemodynamics. Preocclusion heart rate, arterial pressure, and mean left atrial pressure were similar in the two groups of dogs (figure 1). After coronary occlusion, mean left atrial pressure markedly increased, whereas heart rate and arterial pressure did not change appreciably. On reperfusion there was a consistent decrease in arterial as well as in left atrial pressure in both groups. No differences were observed between the two groups during the first 60 min of reperfusion while the drug was being infused.

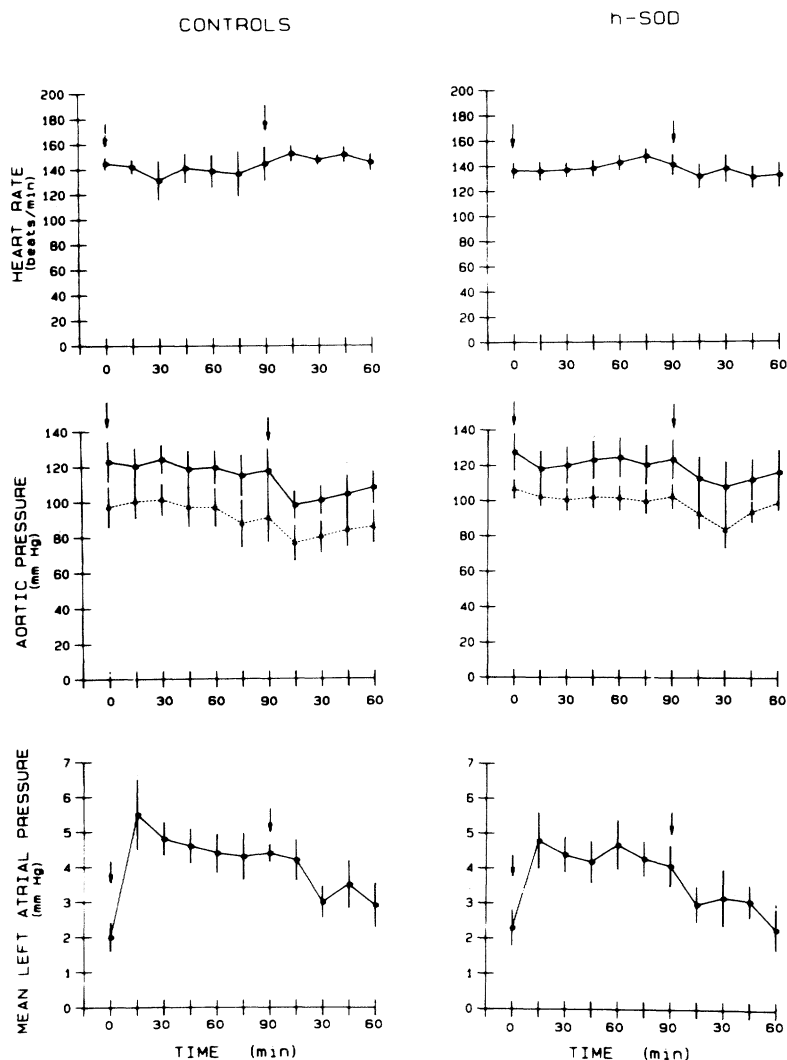


FIGURE 1. Time course of hemodynamic variables in control dogs ($n = 6$, left) and in dogs treated with h-SOD ($n = 8$, right). The arrows mark the onset of ischemia (time zero) and the release of coronary artery occlusion (90 min), respectively. Points represent mean \pm SEM.

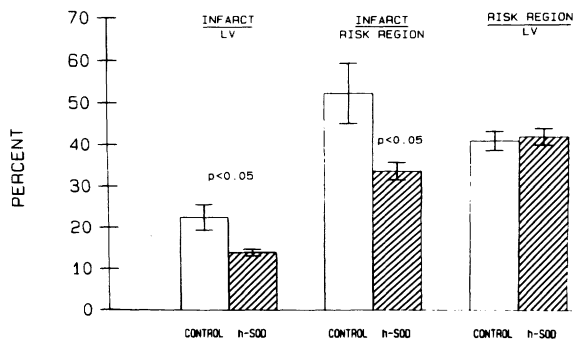


FIGURE 2. Infarct size (expressed as percent of the left ventricle or of the risk region) and average risk region (expressed as percent of the left ventricle) in control (open bars) and h-SOD-treated dogs (hatched bars).

Infarct size. The size of the anatomic vascular area at risk was virtually identical in the two groups. Proximal occlusion of the circumflex artery resulted in an occluded bed size of $40.8 \pm 2.3\%$ of the left ventricle in control dogs and of $41.8 \pm 2.0\%$ in treated dogs (figure 2). In control dogs reperfusion after 90 min was associated with infarction of $22.4 \pm 3.1\%$ of the left ventricle, whereas infarct size was only $13.3 \pm 0.8\%$ of the left ventricle in h-SOD-treated dogs ($p < .05$). When normalized for the extent of the risk region, infarct size averaged $52.2 \pm 7.1\%$ of the risk region in control dogs and $33.6 \pm 2.1\%$ in treated animals ($p < .05$) (figure 2).

Infarcts were not only smaller in the h-SOD-treated group, but the distribution of necrosis within the infarcted area also appeared to be different. Control dogs tended to exhibit confluent infarctions, whereas infarcts from h-SOD-treated animals were often non-confluent, with patchy areas of necrosis interspersed with islands of viable myocardium. Figure 3 shows an example of this finding. It should be emphasized that this is not a "best case" example; the two rings, in fact, were carefully chosen so that the respective risk regions and infarct sizes closely matched the average values in the treatment and control groups. Figure 4 shows that the marked patchiness in h-SOD-treated hearts was not just the result of smaller infarcts in the treated group. In this figure, left ventricular rings demonstrating the same infarct size ($\approx 20\%$ to 25% of risk region) are illustrated for each group. Small infarcts in the control group were still largely confluent, affecting most of the subendocardium in the risk region, whereas in treated dogs areas of nearly transmural necrosis could be found adjacent to spared areas extending throughout the wall to the endocardium.

Histologic study. To rule out the possibility that the smaller infarcts in the treated group were the result of an underestimation of the true extent of necrosis due to

their patchy appearance, representative samples from each heart were also examined histologically. The average area of infarction was $63 \pm 7\%$ by light microscopy and $54 \pm 9\%$ by gross pathology in control hearts and $48 \pm 4\%$ and $44 \pm 4\%$, respectively, in h-SOD-treated hearts. It should be pointed out that these values represent the percentages of necrotic tissue measured in those ventricular rings that were analyzed by both methods and do not represent the infarct size/risk region ratios for the whole left ventricle.

Myocardial blood flow. Baseline flow was 1.28 ± 0.21 ml/min/g in the endocardium and 1.09 ± 0.17 ml/min/g in the epicardium of control dogs and 1.04 ± 0.08 and 1.00 ± 0.10 ml/min/g, respectively, in the h-SOD group. Five minutes after occlusion, collateral blood flow in the center of the ischemic region was very low in both groups, and increased only slightly with time (figure 5). Ten minutes after reperfusion, flow in the subepicardial regions was much higher than that at baseline. This phenomenon was noted in both groups, although it was more pronounced in treated animals. A hyperemic response 234% of that at baseline was also evident in the subendocardial samples from h-SOD-treated dogs, but was virtually absent in control dogs, in which flow averaged 111% of baseline ($p < .05$ vs h-SOD-treated dogs) (figure 5). Finally, flow in the nonischemic region during reperfusion was 1.77 ± 0.29 ml/min/g in the endocardium and 1.60 ± 0.25 ml/min/g in the epicardium of control dogs and 1.37 ± 0.13 ml/min/g in the endocardium and 1.35 ± 0.15 ml/min/g in the epicardium of h-SOD-treated dogs.

The relationship between average collateral blood flow during occlusion and infarct size was also analyzed. Control animals showed the expected inverse relationship between extent of necrosis and amount of collateral blood flow during occlusion, whereas in the treated group there was no correlation (figure 6). This finding was observed when either the subendocardial or the subepicardial flow was analyzed.

Discussion

Since the advent of coronary bypass surgery there have been reports that revascularization of the acutely ischemic myocardium could at times be detrimental.⁶ Experimental studies have suggested that the beneficial effects of restoring the blood supply to the heart might be limited by the occurrence of a specific "reperfusion injury."⁷ The present study shows that administration of the oxygen free-radical scavenger h-SOD at the moment of reflow significantly reduces the extent of myocardial cell necrosis, as compared with that

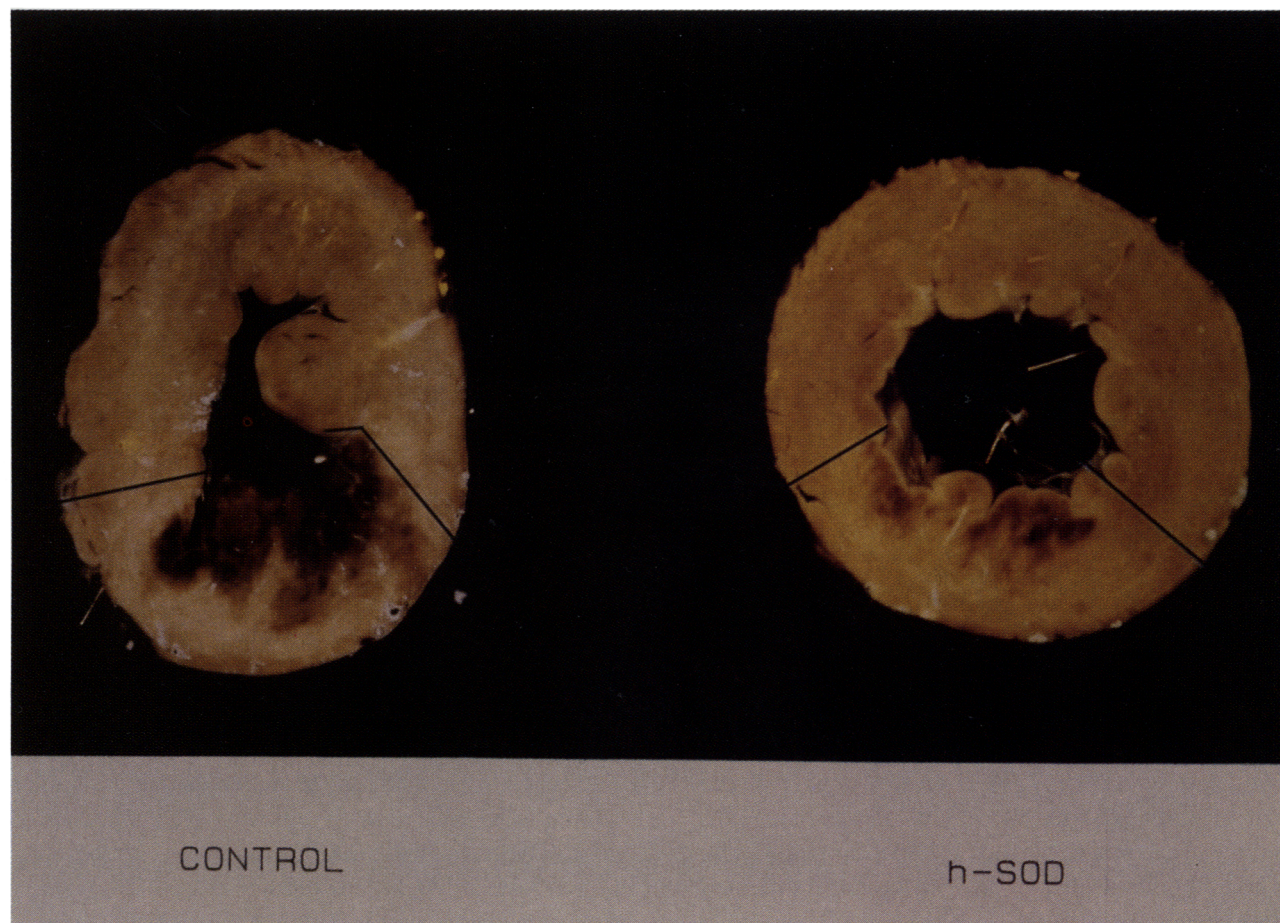


FIGURE 3. The effect of h-SOD treatment is shown in these representative left ventricular rings from one control (*left*) and one h-SOD-treated (*right*) dog. The rings were selected so that risk regions and infarct sizes in each ring were similar to the mean values in the respective treatment groups. The solid lines indicate the boundaries of the risk region. In these unstained, formalin-fixed rings, necrosis appears as the brownish area. The darker spots correspond to areas of hemorrhage. The yellow or pale blue appearance of vessels is due to the postmortem injection of the coronary arteries with colored barium gelatin. The marked reduction in infarct size in the treated heart is evident. Note also that the heart from the control animal exhibits a largely confluent, hemorrhagic infarct, whereas in the treated dog the infarct appears patchy and nonconfluent, with islands of viable tissue interspersed between areas of necrosis.

after reperfusion alone, after experimental coronary artery occlusion. These findings support the hypothesis that specific damage occurs during reflow and that generation of oxygen radicals is a major factor in the genesis of reperfusion injury.^{10–13}

Small quantities of oxygen free radicals are constantly generated in a number of normal biochemical processes. To cope with physiologic amounts of superoxide, the cells possess an enzyme, SOD, that catalyzes the dismutation of this radical to hydrogen peroxide.¹⁸ Acute ischemia, however, profoundly alters this balance by decreasing the cellular levels of SOD¹⁰ while providing the basis for a subsequent “burst” of oxygen radicals at the time of reflow.^{14, 15}

The generation of superoxide anions at reflow is thought to result from oxidation of hypoxanthine,²¹ which accumulates during myocardial ischemia as

ATP is degraded.²² Metabolism of this nucleotide is normally mediated by xanthine dehydrogenase; during ischemia, however, this enzyme is converted to the oxidase form,^{17, 23} which uses oxygen, forming superoxide at the time of reoxygenation. Other possible sources of radicals include the oxidation of catecholamines released locally during ischemia²⁴ and the generation of superoxide anions by activated leukocytes that have migrated into the ischemic tissue.²⁵

The beneficial effect of SOD in the canine preparation of regional ischemia and reperfusion has recently been reported by Jolly *et al.*¹³ These results have been subsequently confirmed by others.^{16, 17} In these experiments, however, the enzyme was administered either before coronary occlusion^{13, 16, 17} or 15 min before reperfusion¹³ and not at the moment of reflow as in the present study. Another difference is that in the pre-

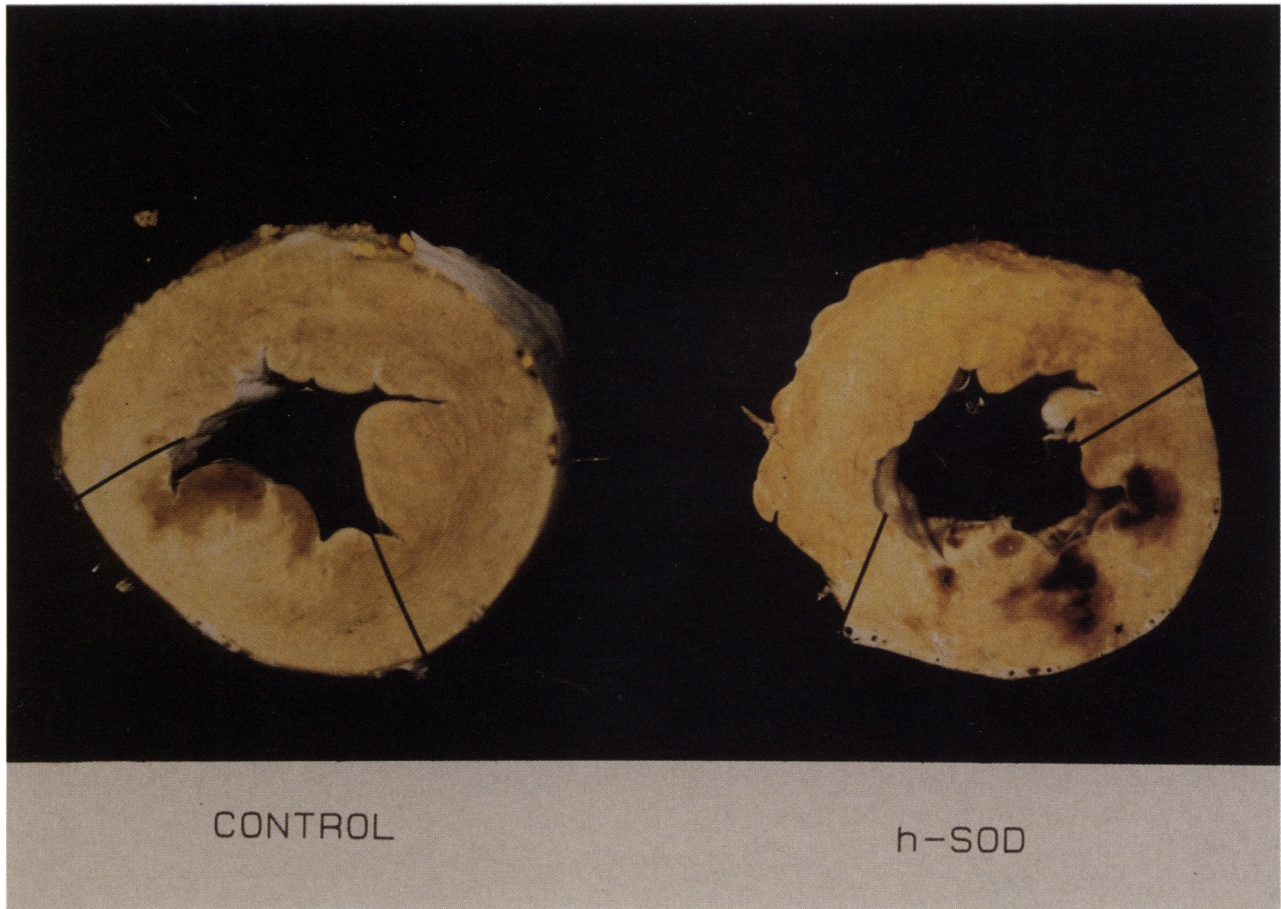


FIGURE 4. Appearance of necrosis in a small infarct from a control dog (*left*) and from a h-SOD-treated animal (*right*). The rings are matched for a similar infarct size/risk region ($\approx 25\%$). Note that the control heart exhibits confluent necrosis confined to the subendocardium, whereas in the h-SOD-treated heart, areas of salvaged subendocardium coexist with zones of almost transmural necrosis.

vious investigations, bovine SOD was used. As a result of recombinant DNA technology, however, the human enzyme (h-SOD) is now available. The enzyme differs from the naturally occurring SOD in human red blood cells only in that its *N*-terminal amino acid is not acetylated.²⁶ Although slight differences in activity or antigenicity of this product versus the native human enzyme could theoretically occur, none has as yet been detected.²⁶

Disagreement has existed as to whether reperfusion actually affects viable tissue, or rather only hastens the death of already irreversibly injured cells.^{4, 5} In our study, control animals undergoing reperfusion after 90 min of occlusion developed nontransmural infarcts that averaged $52.2 \pm 7.1\%$ of the risk region, whereas it has been previously demonstrated that in this preparation a fixed occlusion results in transmural necrosis of about 80% of the anatomic area at risk.³ This suggests that a net beneficial effect was indeed achieved by reperfusion. In h-SOD-treated dogs, however, the

size of the infarcts was even smaller, averaging only $33.6 \pm 2.1\%$ of the risk region ($p < .05$). The finding that an additional 36% of the myocardium at risk of infarcting was salvaged by selective modification of conditions at the time of reflow supports the concept that reperfusion itself can cause death of potentially salvageable tissue.

This study provides several insights into potential mechanisms of myocardial cell necrosis during ischemia and reperfusion. In addition to being smaller, infarcts in h-SOD-treated dogs were often nonconfluent, with islands of viable tissue adjacent to areas of necrosis (figures 3 and 4). Infarcts in control animals, on the other hand, were largely confluent, and the difference between large and small infarcts in this group was essentially due to a greater extension of the necrosis toward the epicardium (figures 3 and 4), in accordance with the notion of the "wave-front phenomenon" of cell death.^{2, 3} The patchy appearance of infarcts in the h-SOD group, which has also been observed in reper-

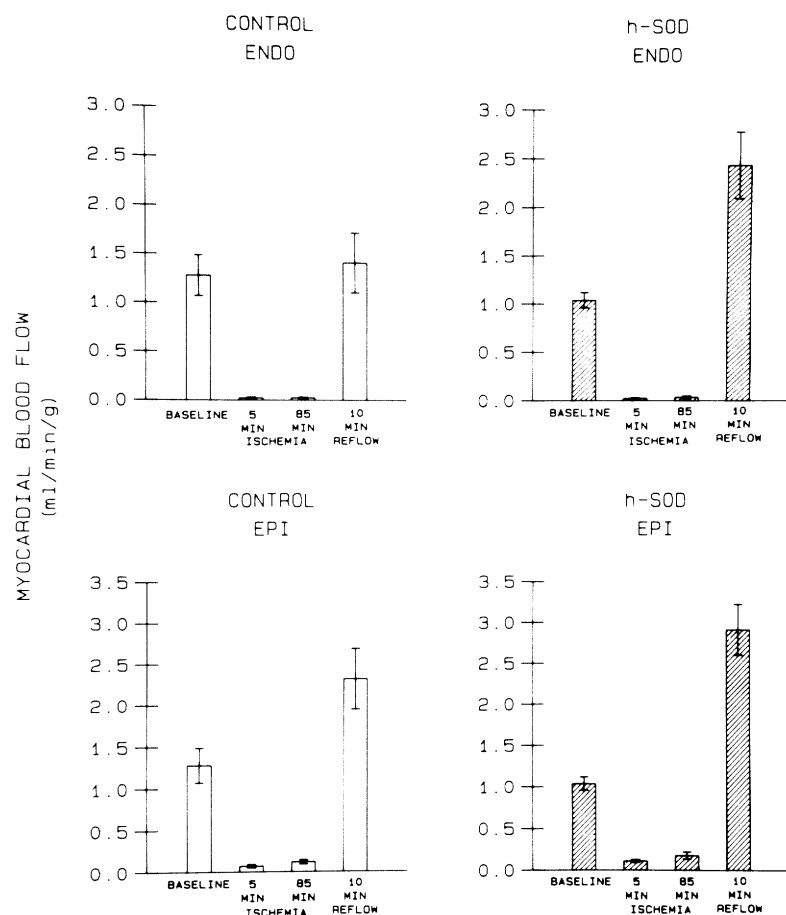


FIGURE 5. Myocardial blood flow at different times during the study in the control (*left*) and the h-SOD group (*right*).

fused dogs treated with SOD and catalase¹³ and in dogs treated with intracoronary perfluorocarbons during reperfusion,²⁷ is strongly suggestive of a vascular distribution of protection from reperfusion injury. Alternatively, this heterogeneity in the spatial distribution of necrosis might be due to a heterogeneous pattern of generation of free radicals during reperfusion.

Although SOD has been shown to reduce reperfusion injury, the concept of a macromolecule crossing an intact sarcolemmal membrane to detoxify cytoplasmic free radicals has been difficult to accept. One answer to this criticism is that the cell membrane of the ischemic myocyte may in fact become permeable. Release of the macromolecules creatine kinase and phosphorylase from ischemic myocytes has recently been demonstrated to occur in the absence of cell necrosis.^{28, 29}

On the other hand, the beneficial effect of h-SOD may be entirely explained by free-radical scavenging within the vasculature rather than within the myocytes. The endothelium appears to represent a primary source of free-radical production in the heart. Immunofluorescence and electron microscopy studies performed in the bovine heart have in fact shown that

xanthine oxidase is found in the cytoplasm of endothelial cells and not in the myocytes.³⁰ The xanthine oxidase activity that has been measured in heart homogenates^{17, 23, 31} or in isolated perfused hearts³¹ may thus derive from the endothelial cells present in the heart sample. Furthermore, it has been demonstrated that perfusion with an enzymatic system that generates free radicals in the vascular lumen is capable of inducing myocardial ultrastructural and functional abnormalities.³² Oxygen radicals generated in the vascular endothelium might thus travel through the vascular wall and directly induce myocyte damage. The possibility that superoxide radicals can cross membranes has indeed been demonstrated both *in vitro*^{33, 34} and *in vivo*.³⁵

SOD-preventable reperfusion injury could also be due to oxygen radicals produced by leukocytes migrating into the ischemic area. A marked reduction in infarct size has, in fact, been shown to result from antibody-induced leukocyte depletion.³⁶ Recent data from Mitsos *et al.*^{37, 38} demonstrated that combined treatment with antineutrophil antiserum and a free-radical scavenger afforded greater reduction of reperfusion injury than either intervention alone. This finding indicates that leukocytes represent a major

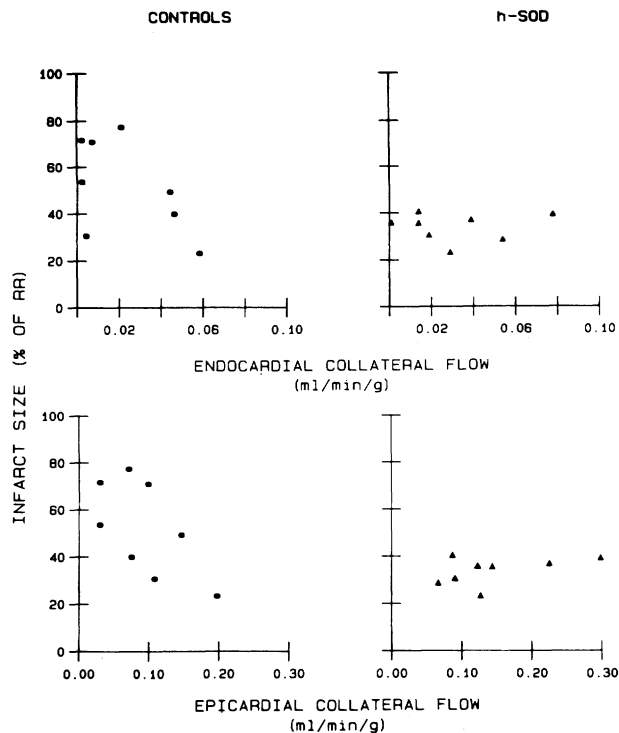


FIGURE 6. Plot of the infarct size 48 hr after coronary artery occlusion (expressed as percent of the risk region) as a function of the collateral flow measured during occlusion in the center of the ischemic region in control dogs (*left*) and in those animals that were subsequently treated with h-SOD during reperfusion (*right*). Points represent the average flow measurements obtained after 5 and 85 min of ischemia in individual animals.

component of reperfusion injury. Other mechanisms, however, must play a role as well, since SOD-preventable reflow damage can be also observed in nonblood perfused hearts.^{12, 39}

Another finding in our study supports the idea of an early vascular involvement in reperfusion damage. In both groups, samples taken from the subepicardial region (which was mostly spared from necrosis) exhibited reactive hyperemia on reperfusion (figure 5). This response was also present in the subendocardium of h-SOD-treated animals, but was absent in control animals. A direct vasodilator effect of h-SOD is unlikely to account for this finding, since flow did not increase in nonischemic myocardium during administration of h-SOD. Higher postischemic flows are presumably a reflection of a lesser degree of cell injury (i.e., "no reflow").⁴⁰ However, it is also possible that this result is the consequence of the prevention or reduction by h-SOD of primary microvascular damage by oxygen free radicals. Whatever the mechanism(s), however, the finding that flow was already impaired as early as 10 min after reperfusion would suggest that at least part of the reperfusion damage occurred within the first

few minutes of reflow. In this respect, results of the only study in vivo bearing on the timing of reperfusion damage indicated that institution of treatment with an oxygen scavenger 40 min after reperfusion did not provide any benefit,¹³ thus suggesting that most of the preventable damage had already occurred by that time. A previous study by Ambrosio and Flaherty³⁹ in isolated, nonblood perfused hearts, on the other hand, showed that a beneficial effect of h-SOD on energy metabolism was already present as early as 5 min after reflow, in accordance with electron paramagnetic resonance studies showing, in a similar preparation, generation of oxygen-centered free radicals within seconds of reperfusion.¹⁵

It has been demonstrated in the canine preparation that the ultimate extent of necrosis is inversely related to the amount of collateral flow during ischemia.^{3, 41} In our study this relationship was present in control hearts, but was strikingly absent in the h-SOD group (figure 6). If one assumes that control dogs exhibited both ischemic and reperfusion damage, while treated dogs showed mainly ischemic damage (reperfusion component eliminated or attenuated), the difference between the two groups should be due to h-SOD-preventable reperfusion injury. By superimposing the regression lines of the epicardial flows in figure 6, one can examine the relationship between severity of ischemia and extent of damage occurring during reperfusion (figure 7). This analysis indicates that in this preparation the extent of reperfusion injury was apparently inversely related to the amount of collateral flow in the ischemic area during occlusion, and was maximal at very low flow and virtually absent in hearts having an epicardial flow of 0.16 ml/min/g or higher. This finding suggests that a relatively high collateral flow that does not protect the heart against ischemic injury may nevertheless prevent or reduce the occurrence of reperfusion-mediated damage. In this study, therefore, the hearts that benefited the most from the intervention were those that had been exposed to the most severe ischemic insult. This is in direct contrast to interventions that protect the myocardium during ischemia, when higher collateral flow has been associated with better protection.⁴² A possible explanation for this phenomenon is that a severe degree of ischemia is required to create the conditions for reperfusion injury. In this respect, it can be postulated that the more severe the ischemia, the greater the accumulation of hypoxanthine (formed from ATP hydrolysis),²² and the greater the depletion of tissue SOD.¹⁰ These factors could both lead to a greater burst of free radicals after reperfusion. Finally, one might speculate that a relatively high flow

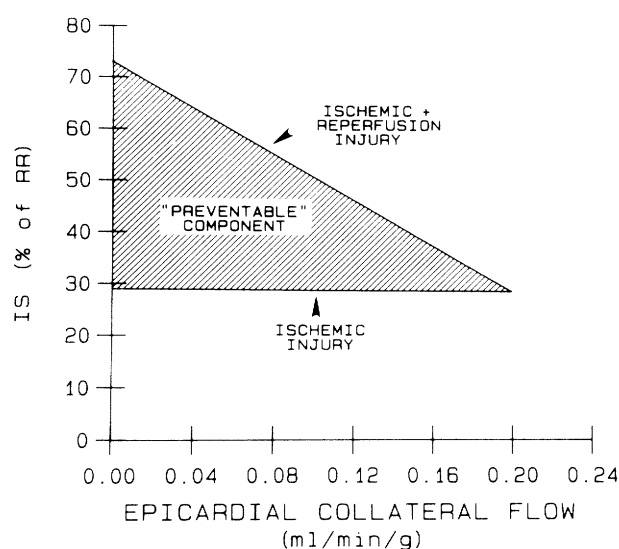


FIGURE 7. Superimposition of the two regression lines from the data in figure 6 relative to the epicardial flows. The upper line represents the correlation between collateral blood flow and infarct size in control animals. Necrosis in this group was the result of ischemic injury combined with reperfusion injury. The lower, flat line refers to the h-SOD-treated group, which might be considered representative of “pure” ischemic injury, since in these dogs the treatment significantly reduced reperfusion injury. The hatched area between the two lines should therefore represent the extent of h-SOD-preventable reperfusion injury as a function of collateral flow during ischemia.

during ischemia may tend to wash out potentially harmful catabolic products (i.e., hypoxanthine, catecholamines, chemotactic factors), thereby reducing or preventing the occurrence of reperfusion injury in that area.

A very recent report by Gallagher *et al.*⁴³ indicates that administration of either SOD or catalase failed to reduce infarct size in a conscious canine preparation of ischemia and reperfusion. Several major differences between our study and Gallagher's could account for this discrepancy. It is likely that there was a much higher delivery of SOD to the myocardium at the crucial moment of reflow in our study, since we used a large left atrial bolus of SOD followed by an intravenous infusion, rather than a continuous intravenous infusion alone. Another important difference lies in the choice of animal preparation. Conscious dogs, as used by Gallagher, have been reported to have smaller infarcts, on the average, than anesthetized dogs after a similar ischemic period.⁴⁴ In Gallagher's study, 3 hr of occlusion in control dogs resulted in infarctions of only 32% of the anatomic risk region, a value considerably smaller than that found by us, as well as by others,^{13, 16} after 90 min of occlusion in anesthetized dogs. In Gallagher's study, collateral blood flow was higher and showed a significant increase over time, while in ours,

it remained virtually unchanged at a much lower level. On the basis of our observation that reperfusion injury was apparently negligible in animals exhibiting a relatively high collateral flow, it might be hypothesized that little reperfusion injury occurred in the dogs in Gallagher's study. Higher collateral flow could explain both the small infarcts in the control dogs and the apparent failure of scavengers to reduce infarct size.

In conclusion, reperfusion of the severely ischemic myocardium results in a component of cellular damage that reduces the beneficial effect of reperfusion itself. This reperfusion-mediated injury is most likely linked to early generation of oxygen free radicals during reflow and is separate from ischemic damage occurring during the period of coronary artery occlusion. Administration of h-SOD at the moment of and during the initial phase of reflow resulted in greater reduction of infarct size compared with that after reperfusion alone. Thus, h-SOD may provide a valuable addition to thrombolytic therapy and/or coronary angioplasty when applied early in patients with acute myocardial infarction.

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