Limitation of Myocardial Infarct Size by Superoxide Dismutase as an Adjunct to Reperfusion After Different Durations of Coronary Occlusion in the Pig

Ulf Näslund, Sören Hägmark, Göran Johansson, Stefan L. Marklund, and Sebastian Reiz

Superoxide dismutase (SOD) has been documented to limit myocardial infarct size in the richly collateralized dog heart. This study was designed to explore this concept in a low-collateralized animal model. A blind, randomized, placebo-controlled protocol was used in 65 pentobarbital-anesthetized pigs subjected to closed-chest left anterior descending coronary artery occlusion for 30 (n = 22), 60 (n = 22), and 90 (n = 14) minutes followed by reperfusion up to 24 hours from the start of occlusion. Another seven control pigs were subjected to 24 hours of permanent occlusion. A total dose of 9 mg/kg bovine CuZn SOD was administered as a bolus injection immediately before reperfusion followed by a 1-hour infusion. Infarct size was assessed by tetrazolium staining. Myocardium at risk and collateral flow were determined by using cerium-141-labeled microspheres (15 μm) during the occlusion. After 30 minutes of occlusion, infarct sizes in placebo versus SOD-treated animals were 45.5±15.7% vs. 23.8±15.6% of myocardium at risk (p=0.007). The corresponding values after 60 minutes of occlusion were 78.6±9.3% vs. 66.9±14.6% (p=0.035). SOD administered after 90 minutes of occlusion did not limit infarct size (88.5±4.8% vs. 92.3±5.2%). Twenty-four hours of coronary occlusion resulted in infarction of 92.4±4.2% of myocardium at risk. (All values are mean±SD.) Ventricular fibrillation occurred in only nine pigs distributed equally between SOD and placebo. The results indicate that CuZn SOD has the potential to further improve the myocardial salvage established by reperfusion of an ischemic pig heart territory. However, the narrow time window for limiting infarct size in the pig by reperfusion is not much extended by SOD. (Circulation Research 1990;66:1294–1301)

Experimental and clinical studies have demonstrated that early reperfusion of the ischemic heart in an evolving myocardial infarction improves survival1,2 and preserves left ventricular performance3 by limitation of the tissue injury.4 Effectiveness of this therapy is, to a great extent, determined by the duration of ischemia preceding the intervention.5

There is evidence that there is a burst of oxygen radical formation after reperfusion of ischemic myocardium6–9 and that administration of antioxidants such as superoxide dismutase (SOD) may limit infarct size.10–14 A majority of the in vivo experimental reperfusion data have been collected in dog models. The results from these studies are conflicting with regard to the effectiveness of SOD in limiting infarct size after a 24-hour or longer experimental protocol.15–18

There are advantages in using the pig heart as opposed to the dog heart for antioxidant therapy studies. The development of collateral flow to the ischemic territory is negligible.19 This simplifies the interpretation of data regarding infarct size. And in contrast to the dog, the pig, like the human heart, contains little or no xanthine oxidase,20,21 an important source of oxygen radicals after reperfusion.10,22

We have previously reported that the combination of CuZn SOD and catalase administered before ischemia and during the first hour of a 5-hour reperfusion period limits infarct size in an open-chest pig model.23 The present closed-chest study in the pig

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was designed to investigate whether SOD alone, given solely at reperfusion, limits infarct size as measured 24 hours after the coronary occlusion. Furthermore, we wanted to establish the therapeutic window for reperfusion and SOD in this model.

Materials and Methods

Randomization Procedures

Fifty-eight pigs were randomized into six groups to receive SOD or placebo after 30, 60, or 90 minutes of coronary occlusion. Twenty-two of the animals were assigned to 30 minutes of coronary occlusion, another 22 animals were assigned to 60 minutes of occlusion, and 14 animals to 90 minutes of occlusion. The number of pigs in the 90-minute group was smaller because pilot experiments had demonstrated an infarction comprising approximately 90% of the ischemic myocardium at risk with a small standard deviation. As a separate unrandomized control group to reperfusion therapy, a sustained occlusion was performed for 24 hours (no reperfusion) in another seven animals.

The treatment code was disclosed after all calculations and exclusions were completed.

Preparation of the Animal

Female Swedish landrace pigs (mean weight, 32.1 kg; range, 27–37 kg) were premedicated with acepromazine (25 mg i.m.). Anesthesia was induced with sodium pentobarbital (20 mg/kg i.v.) followed by a continuous infusion at around 0.2 mg/kg/min throughout the experiment. Body temperature was monitored and kept at approximately 38°C with electric pads. After tracheostomy, the animals were mechanically ventilated by a volume-cycled ventilator (ServoVent 900 B, Siemens Elema, Stockholm, Sweden) at \( \text{FiO}_2 = 0.3 \). A single lateral chest-lead ECG was recorded continuously. During the occlusion period and the first 2 hours of the reperfusion period, a 12-lead ECG was used for confirmation of myocardial ischemia and reperfusion. The pigs were heparinized with 3,500 IU i.v. during the preparation. Hemodynamic monitoring and blood sampling were performed after cutdown via catheters introduced into the right femoral, right subclavian, and pulmonary arteries.

A modified coronary angiography catheter was advanced under fluoroscopy into the left descending coronary artery (LAD) via the right internal carotid artery. A Teflon-coated lead ball (\( \phi = 2.0 \) mm) attached to a monofilament nylon thread (Novafil 7-0, Davis and Geck, Wayne, New Jersey) was floated into the LAD. The position of the ball was repetitively controlled by fluoroscopy. The criterion for a stable position was that the ball was permanently located in a similar distance and angle to a metal marker placed externally on the thoracic wall.

When the coronary occlusion was achieved and ischemic electrocardiographic changes were observed, \( 15 \times 10^6 \) cerium-141 radioactively labeled microspheres (15 \( \mu \)m, Du Pont Scandinavia AB, Stockholm, Sweden) were injected via a catheter in the apex of the left ventricle for delineation of the ischemic zone in postmortem autoradiograms. The position of the catheter was checked by fluoroscopy and pressure monitoring. Reperfusion of the ischemic myocardium was achieved by slowly retracting the ball by the attached thread, back to the tip of the guide catheter, placed in the ascending aorta.

Benzylenicillin (3 g i.v.) was administered approximately 3 hours into the reperfusion period. No antiarrhythmical or other drugs were permitted in the protocol.

The study was approved by the Swedish Committee for care and use of laboratory animals in medical research.

Preparation of Superoxide Dismutase and a Stained Placebo Solution

Bovine CuZn SOD (courtesy of Pharmacia AB, Uppsala, Sweden) was dissolved in 10 mM potassium phosphate (pH 7.40) in 0.15 M NaCl to a final concentration of 10 mg/ml. The specific activity of the bovine CuZn SOD was \( 159 \times 10^3 \) units/mg. The endotoxin content was negligible. The buffer was used as the placebo solution. Because CuZn SOD is blue-green, the placebo solution had to be stained to comply with the needs of a blinded study. Evans blue (1.3 mg/l) plus tartrazine (5.1 mg/l) in the buffer solution made it indistinguishable from the CuZn SOD solution.

Treatment Protocol

Five minutes before the start of the reperfusion period, 20 ml SOD (10 mg/ml) or placebo was injected at 4 ml/min. The bolus injection was followed by a continuous infusion of SOD (100 mg/hr) or placebo over the next 60 minutes. In the control group with a 24-hour, sustained occlusion, neither SOD nor placebo was given.

Infarct Size, Myocardium at Risk, and Collateral Flow

Animals were killed 24 hours after the start of the occlusion period by an overdose of sodium pentobarbital. After rapid excision of the heart, the atria were cut off and the ventricles were frozen (\(-70^\circ\) C). The heart was sectioned perpendicular to the apex-base axis in 4-mm thin slices, which were weighed.

Myocardial infarct size was quantified by using a standard triphenyltetrazolium chloride method.\(^{24}\) The slices were immersed for 20 minutes in 0.8% triphenyltetrazolium chloride (Sigma Chemical, St. Louis, Missouri) dissolved in 0.1 M Tris HCl buffer (pH 8.0) at 30°C. Viable myocardium could be delineated from the unstained, infarcted myocardium by its bright red appearance. These delineation borders were traced on transparent sheets for subsequent planimetry.

Myocardial at risk was defined in postmortem autoradiograms. The slices were placed on x-ray films (Singul-XRP, CEA-verken, Strängnäs, Sweden),
TABLE 1. Excluded Animals

<table>
<thead>
<tr>
<th>Pig</th>
<th>Occlusion time (minutes)</th>
<th>Treatment</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>90</td>
<td>Placebo</td>
<td>Left main stem dissection</td>
</tr>
<tr>
<td>19</td>
<td>30</td>
<td>Placebo</td>
<td>Poor TTC staining</td>
</tr>
<tr>
<td>25</td>
<td>60</td>
<td>Placebo</td>
<td>Left main stem dissection</td>
</tr>
<tr>
<td>34</td>
<td>30</td>
<td>SOD</td>
<td>Catheter-induced thrombosis</td>
</tr>
<tr>
<td>37</td>
<td>60</td>
<td>SOD</td>
<td>Catheter-induced thrombosis</td>
</tr>
<tr>
<td>52</td>
<td>90</td>
<td>SOD</td>
<td>Technical problem with autoradiography</td>
</tr>
<tr>
<td>53</td>
<td>90</td>
<td>Placebo</td>
<td>Incomplete coronary occlusion</td>
</tr>
<tr>
<td>56</td>
<td>90</td>
<td>Placebo</td>
<td>Catheter-induced thrombosis</td>
</tr>
<tr>
<td>57</td>
<td>90</td>
<td>SOD</td>
<td>Anaphylactic shock—penicillin</td>
</tr>
</tbody>
</table>

TTC, triphenyltetrazolium chloride; SOD, superoxide dismutase.

which were exposed for 48 hours at −20°C. Myocardium perfused during the coronary occlusion could be distinguished by the distribution of the emission from 141Ce-labeled microspheres. Myocardium at risk was measured by planimetry of the autoradiograms. Volumes of myocardium at risk and the infarcted tissue were determined with the method described by Boor and Reynolds.25

In the animals with 30 and 60 minutes of occlusion, sections of slices, comprising normal myocardium and myocardium at risk, were excised to allow determination of subendocardial, midmyocardial, and subepicardial blood flow as described by Heymann et al.26 From these data, collateral flow to the ischemic territory could be calculated.

**Hemodynamic Measurements**

Heart rate was derived from the continuously recorded ECG. Systemic blood pressure was recorded via the femoral artery or the aorta throughout the experiment. Left ventricular end-diastolic pressure was recorded via the catheter used for the microsphere injection. All pressure recordings were made via pressure transducers (SensoNor 840, AME, Horten, Norway) on a Mingograf 7 (Siemens-Elema).

**Plasma SOD Analysis**

SOD was determined by its ability to catalyze the disproportionation of O2·− in alkaline (pH 9.50) aqueous solution. This reaction was studied directly in a spectrophotometer.27 One unit is defined as the SOD activity that brings about a disproportionation of the superoxide radical at a rate of 0.1/sec in a 3-ml reaction volume. The method is very sensitive. One unit in the commonly used original xanthine oxidase–cytochrome c assay28 corresponds to 40 units in the present assay.

**Statistical Analyses**

Mean values of hemodynamic parameters, collateral flow, measurements of the size of myocardium at risk, and the infarcted tissue were compared between placebo- and CuZn SOD–treated groups and between placebo-treated groups with different durations of coronary occlusion by Wilcoxon’s rank sum test. A value of p<0.05 was regarded as statistically significant. All values were expressed as mean±SD.

**Results**

**Exclusions and Complications**

Nine of the 65 animals (14%) were excluded for reasons presented in Table 1. In five of these, the size of myocardium at risk could be determined. No difference in infarct size was seen between these animals and those included for analysis.

Ventricular fibrillation (VF) occurred in nine animals (Table 2), and with the exception of three animals excluded from the study because of catheter complications, external defibrillation was immediately successful. Four of the remaining six pigs that developed VF did so 15–20 minutes into the occlusion period. In the remaining two, both assigned to 30 minutes of coronary occlusion, VF occurred during the first minutes of reperfusion. One of these was treated with SOD.

**Infarct Size and Collateral Flow**

The size of the infarcted tissue within myocardium at risk increased with increasing duration of ischemia (Figure 1, Table 3). In placebo-treated animals, the infarct/risk zone ratio was significantly lower in the

**TABLE 2. Ventricular Fibrillation**

<table>
<thead>
<tr>
<th>Pig</th>
<th>Occlusion time (minutes)</th>
<th>Treatment</th>
<th>VF occlusion</th>
<th>VF reperfusion</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>90</td>
<td>Placebo</td>
<td>X</td>
<td></td>
<td>Excluded</td>
</tr>
<tr>
<td>24</td>
<td>60</td>
<td>Placebo</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>60</td>
<td>Placebo</td>
<td>X</td>
<td></td>
<td>Excluded</td>
</tr>
<tr>
<td>29</td>
<td>30</td>
<td>Placebo</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>60</td>
<td>Placebo</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>30</td>
<td>SOD</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>46</td>
<td>60</td>
<td>SOD</td>
<td>X</td>
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<td></td>
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<tr>
<td>51</td>
<td>90</td>
<td>Placebo</td>
<td>X</td>
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</tr>
<tr>
<td>56</td>
<td>90</td>
<td>Placebo</td>
<td>X</td>
<td></td>
<td>Excluded</td>
</tr>
</tbody>
</table>

VF, ventricular fibrillation; SOD, superoxide dismutase.
group with 30 minutes of occlusion than in pigs with 60 minutes of occlusion (45.5 ± 15.7 vs. 78.6 ± 9.3, p < 0.001). Placebo-treated pigs with 60 minutes of occlusion appeared to develop smaller infarcts than the placebo-treated animals with 90 minutes of occlusion (p = 0.036) or 24 hours of occlusion (p = 0.001). There was no difference in infarct size between placebo-treated pigs with 90 minutes and 24 hours of occlusion. The therapeutic window for reperfusion per se appears to close after 60 minutes of coronary occlusion. SOD, as adjunct to reperfusion, did not extend the therapeutic window, but this combination therapy further limited the tissue injury. A highly significant protective effect was seen in animals with a 30-minute occlusion. A statistically significant, but less pronounced, myocardial salvage was seen in SOD-treated animals with 60 minutes of occlusion.

The infarcted tissue, after 30 minutes of coronary occlusion, was irregular and spotty, whereas the picture was homogeneous in subendocardial and midmyocardial regions of animals with 60 minutes of coronary occlusion. In pigs with 90 minutes of occlusion, a homogeneous transmural injury was seen.

Myocardial at risk was smaller in placebo-treated pigs with 30 minutes of coronary occlusion (Table 3). When the amount of myocardium at risk was related to the size of the hearts, no statistical difference was seen between the two groups.

As expected, the collateral flow was very low during the early phase of the occlusion period. The calculated transmural mean flow in percent of flow in nonischemic regions of the myocardium ranged in the groups from 0.8% to 1.6%, and no statistically significant differences were seen between the treatment groups. In two of the placebo-treated pigs, a higher collateral flow was seen for unknown reasons. These animals developed infarcts with sizes close to the mean of the groups. In this study, the collateral flow was too low to make reliable correlations to calculated infarct size.

### Table 3. Infarct Size and Myocardium at Risk

<table>
<thead>
<tr>
<th></th>
<th>30-min Occlusion</th>
<th>60-min Occlusion</th>
<th>90-min Occlusion</th>
<th>24-hour Occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SOD (n=10)</td>
<td>Placebo (n=10)</td>
<td>SOD (n=10)</td>
<td>Placebo (n=10)</td>
</tr>
<tr>
<td>HW (g)</td>
<td>120.6±23.2</td>
<td>113.0±10.7</td>
<td>119.4±13.8</td>
<td>121.3±11.2</td>
</tr>
<tr>
<td>INF (cm³)</td>
<td>2.3±1.0</td>
<td>2.8±1.7</td>
<td>6.1±2.4</td>
<td>6.1±1.8</td>
</tr>
<tr>
<td>MAR (cm³)</td>
<td>8.7±2.6*</td>
<td>6.0±2.1</td>
<td>9.0±2.2</td>
<td>7.6±1.8</td>
</tr>
<tr>
<td>INF/MAR (cm³/g×100)</td>
<td>1.9±1.6</td>
<td>2.5±1.7</td>
<td>5.1±1.9</td>
<td>5.1±1.7</td>
</tr>
<tr>
<td>MAR/HW (cm³/g×100)</td>
<td>7.3±2.1</td>
<td>5.4±2.1</td>
<td>7.6±2.0</td>
<td>6.4±1.9</td>
</tr>
<tr>
<td>INF/MAR (%)</td>
<td>23.8±15.6†</td>
<td>45.5±15.7</td>
<td>66.9±14.6*</td>
<td>78.6±9.3</td>
</tr>
</tbody>
</table>

All values are mean ± SD. SOD, superoxide dismutase; HW, heart weight; INF, infarcted tissue volume; MAR, myocardium at risk volume; INF/HW, infarcted tissue volume/heart weight; MAR/HW, myocardium at risk volume/heart weight; INF/MAR, infarcted tissue volume/myocardium at risk volume.

* p = 0.035 compared with placebo.
† p = 0.007 compared with placebo.
Hemodynamic Measurements

There was no difference in the metabolic demand, expressed as the rate pressure product, left ventricular end-diastolic pressure early in the occlusion period, and the body temperature between SOD- and placebo-treated animals (Table 4).

Plasma SOD Activity

Figure 2 shows the plasma SOD activity resulting from the SOD bolus injection and infusion. During the first reperfusion hour the activities were very high and about as high as the intracellular SOD activity in the porcine myocardium.29 The enzyme was then rapidly lost. At 6 hours, the activity was six times higher than the basal plasma SOD activity and about 5% of the peak SOD activity.

Discussion

The most important result of this investigation carried out in a porcine closed-chest preparation was the limitation of myocardial infarct size induced by CuZn SOD given as an adjunct at reperfusion. The myocardial salvage was demonstrated 24 hours after ischemia was induced. Reperfusion per se was effective in limitation of tissue injury, but CuZn SOD further limited infarct size. The therapeutic window of both reperfusion and CuZn SOD was, however, narrow. No effect of treatment was observed if the ischemic period exceeded 60 minutes. The findings support the hypothesis that oxygen radicals are injurious when the ischemic myocardium is rapidly reperfused.

There are important differences between the design of the present study and earlier studies. First, it was blind and placebo controlled. The placebo buffer solution was stained to look exactly like the CuZn SOD solution. We think this is important, because the animal preparation is complicated and many interventions are made in the course of the experiments. Furthermore, after short ischemic periods (30 minutes) in particular, the infarcts are spotty and a certain degree of subjectivity is unavoidable in the delineation of infarcted areas.

Second, a low-collateralized pig model was used. In reperfusion studies, the porcine heart offers advantages, at least theoretically. The distribution of the coronary arteries mimics the human heart.30

Corrections for collateral flow in the interpretation of infarct size data, when using a pig heart model, are not necessary because acute collateralization is negligible. The pig heart, similar to the human heart30 but unlike the dog heart,10 has a low content of

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**TABLE 4. Hemodynamic Parameters and Body Temperature**

<table>
<thead>
<tr>
<th></th>
<th>SOD</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>Preocclusion 121 ± 15 (n=25)</td>
<td>130 ± 20 (n=23)</td>
</tr>
<tr>
<td></td>
<td>15-min occlusion 130 ± 20 (n=25)</td>
<td>131 ± 22 (n=23)</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>Preocclusion 93 ± 15 (n=16)</td>
<td>90 ± 13 (n=17)</td>
</tr>
<tr>
<td></td>
<td>15-min occlusion 94 ± 13 (n=22)</td>
<td>89 ± 14 (n=22)</td>
</tr>
<tr>
<td>RPP (beats/min×mm Hg)</td>
<td>Preocclusion 14,300 ± 2,600 (n=25)</td>
<td>15,300 ± 3,300 (n=23)</td>
</tr>
<tr>
<td></td>
<td>15-min occlusion 14,900 ± 3,200 (n=25)</td>
<td>14,400 ± 3,300 (n=23)</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>Preocclusion 10.5 ± 2.4 (n=20)</td>
<td>9.6 ± 2.2 (n=20)</td>
</tr>
<tr>
<td></td>
<td>10-min occlusion 11.1 ± 2.4 (n=15)</td>
<td>9.9 ± 2.2 (n=17)</td>
</tr>
<tr>
<td>Body temp (°C)</td>
<td>Preocclusion 38.0 ± 0.8 (n=25)</td>
<td>38.0 ± 1.0 (n=24)</td>
</tr>
</tbody>
</table>

All values are mean±SD. SOD, superoxide dismutase; HR, heart rate; MAP, mean arterial pressure; RPP, rate pressure product; LVEDP, left ventricular end-diastolic pressure; Body temp, body temperature.

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**Figure 2.** Time course of plasma superoxide dismutase (SOD) activity. Plasma SOD activity resulting from a 200-mg bolus injection given intravenously immediately before coronary reperfusion and followed by a 60-minute continuous intravenous infusion of 100 mg bovine CuZn SOD. The SOD activities were analyzed in 6 animals with 60 minutes of occlusion and are presented as the mean±SD. The mean basal plasma SOD activity of the pigs, mainly given by extracellular SOD, was 103±23 units/ml.
xanthine oxidase.\textsuperscript{31} Thus, in the ischemic, but via collaterals, partly perfused xanthine oxidase–rich canine heart, a production of oxygen radicals might occur during occlusion. Furthermore, in the reperfusion phase, a mixture of complete, partial, and no reflow to the ischemic territory might conceivably cause a prolonged production of hypoxanthine, resulting in a sustained oxygen radical formation. It is of interest that when different animal models are discussed, the basal plasma SOD activity resulting from the extracellular superoxide dismutase differs greatly between species. The mean activity in dogs, humans, pigs, rats, and rabbits is 2, 18, 85, 350, and 850 units/ml, respectively.\textsuperscript{32} Thus, the pathogenetic importance of superoxide radicals in the extracellular compartment might vary considerably between species. Possibly, the effect of intravenous SOD therapy is greatest in dogs, followed by humans, pigs, rats, and rabbits, in that order.

Third, this study was performed in a closed-chest preparation in lightly anesthetized animals. The pig is extremely sensitive to psychological and surgical stress, which results in hemodynamic instability and ventricular arrhythmias. The combination of the anesthetic procedures and the avoidance of major surgical trauma during the preparation provided conditions of hemodynamic stability and a low complication rate. The experimental mortality was 3/65 (4.5\%) and was related to catheter manipulation and one anaphylactic reaction to penicillin. Compared with our previous open-chest preparation\textsuperscript{23} and with open-chest models of others,\textsuperscript{33} there was a remarkable reduction in the number of animals that developed ventricular fibrillation (Table 2). The low number of dropouts with our preparation makes the interpretation of data more reliable.

Myocardial salvage by CuZn SOD in dogs has been shown to occur after short reperfusion periods.\textsuperscript{10,11} On the other hand, Przyklenk and Klener\textsuperscript{34} did not verify this in a recently published study with an experimental protocol of 6 hours. With 24-hour or longer reperfusion periods, some studies have indicated a protective effect.\textsuperscript{12–14,35} However, the majority of investigations fail to confirm this.\textsuperscript{15–18} The myocardial salvage of CuZn SOD in dogs is therefore still clouded in controversy. The reason for the discrepancies is not clear. Measurements with spin-traps\textsuperscript{7–9} indicate that the production of oxygen radicals peaks within 5 minutes postreperfusion. A high plasma SOD activity during the early reperfusion phase should therefore be essential. This view is supported by results of a comparison between different dosage schedules.\textsuperscript{35} We achieved very high postreperfusion levels of SOD activity. Because of lack of data, our results cannot be compared with some of the studies presented earlier. The plasma SOD activity was about four times higher than in a canine study in which no significant effect of treatment with SOD could be demonstrated.\textsuperscript{17} However, our results differ from the recently published and extensive study by Nejima and coworkers,\textsuperscript{18} who used both recombinant SOD and the combination of SOD and catalase. The dose and prolonged administration of SOD during the first hour of the reperfusion was fairly similar to the treatment protocol in the present study. In another 24-hour ischemia-reperfusion study in the pig, with a 45-minute occlusion period, no significant effect of CuZn SOD treatment was found.\textsuperscript{33} The SOD was given in a “low dose (0.66 mg/kg)” as a 45-minute intracoronary infusion during the first part of the reperfusion. Although not measured in the study, the effective concentration during the infusion would probably be high, but would fall precipitously thereafter.

A problem in treatment with CuZn SOD is the very short plasma half-life. Plasma CuZn SOD activity has a half-life of 7 minutes in rodents\textsuperscript{36} and 20–30 minutes in larger mammals such as pigs\textsuperscript{23} and humans.\textsuperscript{36} There is a rapid fall in SOD activity after the infusion is discontinued. (Figure 2). Even after a short (15-minute) period of ischemia in the dog, increased oxygen radical formation could be demonstrated during the whole 3-hour period of the experiment.\textsuperscript{9} Studies with the isolated perfused rat heart reveal that the intensity of oxygen radical production is proportional to the duration of ischemia.\textsuperscript{8} It is therefore likely that oxygen radical formation is increased over many hours postreperfusion in studies of infarct size limitation. Part of the difference between the short- and long-term reperfusion studies might be due to continued oxygen radical production and the rapid loss of plasma CuZn SOD activity. This problem may be greater in the xanthine oxidase–rich canine heart than it is in the pig heart, which would explain the differences between some canine studies and ours. In a recently published study in the dog with long-acting polyethylene glycol–substituted CuZn SOD, a significant salvage (estimated after 4 days) was achieved, which may support this hypothesis.\textsuperscript{14}

The sources of superoxide and mechanisms for the development of tissue injury are not well understood. Xanthine oxidase action on hypoxanthine, formed by ischemia-induced adenine nucleotide degradation,\textsuperscript{20} may be a less important source in the pig. Much attention has been focused on the role of neutrophil leukocytes, which accumulate in the injured myocardium.\textsuperscript{37–40} Attenuation of tissue injury has been reported after administration of antineutrophil serum\textsuperscript{40} and drugs that inhibit neutrophil accumulation.\textsuperscript{37–39} The neutrophils might cause injury by reactive oxygen intermediates or by other products released by the activation of the cells as well as by obstruction of capillary flow.\textsuperscript{37} SOD might protect the heart against direct toxic effects of the oxygen intermediates and by reducing the formation of superoxide-induced factors, which are chemotactic for neutrophils.\textsuperscript{41,42}

Iron-sulfur proteins, catecholamines, flavins, and quinones accumulate in their reduced form during ischemia. Reoxygenation may result in a burst of oxygen radicals produced at autooxidation of these
substances. Superoxide is apparently released from endothelial cells in the brain during synthesis of prostaglandins and leukotrienes. A similar mechanism might be operative in reperfusion of the heart. Superoxide and other reducing substances can release iron from ferritin. Increased amounts of reactive iron at the time of reperfusion may then augment both formation and toxic effects of oxygen radicals.

Endothelium-derived relaxing factor (EDRF) is highly sensitive to superoxide radicals, and SOD prolongs its duration of action. EDRF relaxes vessels and reduces platelet adhesion. Oxygen radicals may directly inactivate EDRF and reduce its production via endothelial injury. Such mechanisms could possibly contribute to reduced postischemic myocardial blood flow. Treatment with SOD plus catalase has been reported to preserve the cardiac microvasculature and improve regional blood flow in the dog after 2 hours of occlusion and 4 hours of reperfusion.

The thromboxane synthetase inhibitor CGS-13080 together with tissue-type plasminogen activator markedly limited infarct size in a regional ischemia-reperfusion model in the cat. Although the mechanism of the myocardial salvage is not quite clear, the findings may indicate the importance of preserving the microvasculature and of influencing the balance in the vasculature; ideally achieving a state of vasodilation and low platelet aggregation.

Finally, decreased myocardial content of antioxidants like SOD, catalase, and glutathione peroxidase has been observed during ischemia and reperfusion. This would make the myocardium more susceptible to oxygen radicals at reperfusion and partly explain why high concentrations of antioxidants could limit injury in the reperfused territory.

Disturbing factors in oxygen-radical research are 1) the lack of knowledge concerning the pathogenesis of reperfusion injury and 2) the key sources of oxygen radicals as well as the optimal dose and duration of the SOD treatment are not known. Thus, the beneficial effect of CuZn SOD in this study has no evident explanation. It would appear that the concept of SOD as an adjunct to reperfusion procedures deserves further basic experimental study before clinical trials are started.

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