EXPERIMENTAL STUDIES

Low Doses of Superoxide Dismutase and a Stable Prostacyclin Analogue Protect in Myocardial Ischemia and Reperfusion

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The effects of low dose human superoxide dismutase and low dose taprostene, a stable analogue of prostacyclin, were investigated separately and together in a model of myocardial ischemia (1.5 h) with reperfusion (4.5 h) in open chest, anesthetized cats. Taprostene (60 mg/kg per min), human superoxide dismutase (0.25 mg/kg per h), both agents together, or their vehicle, were infused intravenously to cats starting 0.5 h after occlusion of the left anterior descending coronary artery. Neither low dose taprostene nor low dose human superoxide dismutase exerted any endothelial or myocardial protection in this model. However, the two agents together showed a significant endothelial and myocardial protection in cats with myocardial ischemia and reperfusion. Compared with cats that were untreated or received only taprostene or human superoxide dismutase, cats receiving both agents exhibited a lower plasma creatine kinase activity at every time point observed after reperfusion, a reduced area of cardiac necrosis (7 ± 2% vs. 21 ± 5% area at risk, p < 0.001), lower myocardial lactate dehydrogenase activity in the ischemic region (p < 0.01) and a significant preservation of vasorelaxant responses of left anterior descending coronary rings to endothelium-dependent vasodilators, acetylcholine (p < 0.001) and A-23187 (p < 0.001). Taprostene appears to act additively with human superoxide dismutase to inhibit neutrophil adherence and activation and to inactivate superoxide radicals, and thus reduce cell injury 4.5 h after reperfusion of the ischemic heart. Use of this agent may allow low doses of superoxide dismutase to be used more effectively in early myocardial ischemia.

Methods

Animal preparation. Adult male cats (2.6 to 3.3 kg) were anesthetized with sodium pentobarbital (30 mg/kg body weight, intravenously). An endotracheal cannula was inserted through a midline incision, and all cats were placed on intermittent positive pressure ventilation (Harvard small animal respirator). Polyethylene catheters were inserted into the right external jugular and the right femoral veins for...
infusion of additional sodium pentobarbital, drugs or their vehicle. The right femoral artery was also cannulated and connected to a Statham P23AC pressure transducer (Gould) to record mean arterial blood pressure electronically. A midsternal thoracotomy was performed and the pericardium was opened. A 2-0 silk ligature was placed around the left anterior descending coronary artery to 10 mm from its origin. Standard lead II of the scalar electrocardiogram (ECG) was used to determine heart rate and ST segment elevation. The ECG and mean arterial blood pressure were continuously recorded on a Grass model 7 oscillographic recorder. The pressure-rate index, an approximation of myocardial oxygen demand, was calculated as the product of mean arterial blood pressure and heart rate divided by 1,000.

**Experimental protocol.** After completion of all surgical procedures and a 30-min stabilization period, the cats underwent a baseline reading of heart rate and mean arterial blood pressure; the initial blood sample was obtained at this time. Myocardial ischemia (defined as time 0) was produced by tightening the previously placed reversible ligature around the left anterior descending coronary artery to completely occlude the vessel. After 1.5 h of ischemia, the ligature was untied and the ischemic myocardium was reperfused for 4.5 h, resulting in a total observation period of 6 h. Thirty minutes after coronary occlusion, taprostene (pH 9) (Grunenthal GmbH), human superoxide dismutase (3.109 superoxide dismutase, protein, Grunental GmbH), the two agents together, or their vehicle, were given as a constant infusion for the remainder of the experiment (5.5 h total). In previous experiments (9,15) using the same cat model of myocardial ischemia and reperfusion, we have demonstrated that infusion of 100 ng/kg per min taprostene or 5 mg/kg per h human superoxide dismutase separately produced significant endothelial and cardiac protection. To obtain an infusion rate that produced a marginal amount of myocardial protection when used alone, we initially employed a variety of infusion rates of taprostene (20 to 80 ng/kg per min) or human superoxide dismutase (0.1 to 1 mg/kg per h). An infusion rate of 60 ng/kg per min taprostene and 0.25 mg/kg per h human superoxide dismutase was eventually chosen. Administration of human superoxide dismutase or taprostene separately at a higher dose protected against myocardial injury, but combined administration of these agents at a lower dose did not exert full protection. The cats were randomly separated into four groups subjected to myocardial ischemia: group a, myocardial ischemia + vehicle (n = 6); group b, myocardial ischemia + taprostene (n = 7); group c, myocardial ischemia + human superoxide dismutase (n = 6); and group d, myocardial ischemia + taprostene + human superoxide dismutase (n = 6). Additional cats were subjected to a sham myocardial ischemia procedure in which all procedures were identical to those in the cats subjected to myocardial ischemia except that the ligature around the left anterior descending coronary artery was not tightened. This model of myocardial ischemia in the cat has been previously shown to have very low collateral blood flow in the necrotic region (17). At 5 h after coronary ligation, myocardial blood flow determined with 15-μm microspheres was found to be 5% of control coronary flow (17).

**Plasma creatine kinase (CK) analysis.** Arterial blood samples (2 ml) were drawn immediately before ligation and hourly thereafter. The blood was collected in polyethylene tubes containing 200 IU of heparin sodium. Samples were centrifuged at 2,000 × g and 4°C for 20 min and the plasma was removed for biochemical analysis. Plasma protein concentration was assayed with the biuret method (18) and plasma CK activity was measured with the method of Rosalki (19) and expressed as IU/mg protein × 10⁻³.

**Myocardial tissue analysis.** At the end of the 6 h experimental period, the ligature around the left anterior descending coronary artery was retightened. Then 30 ml of 0.5% Evans blue dye was injected into the left atrium to stain the area of the myocardium that was perfused by the patent coronary arteries. The area at risk was thus determined by negative staining. The heart was excised rapidly and placed in warmed, oxygenated Krebs-Henseleit buffer. The left circumflex and left anterior descending coronary arteries were isolated and removed for subsequent study of coronary ring vasoreactivity. The right ventricle and great vessels were removed and the left ventricle was sliced parallel to the atrioventricular groove in 3-mm thick sections. The unstained portion of the myocardium (that is, the total area at risk) was separated from the Evans blue-stained portion of the myocardium (that is, the area not at risk). The area at risk was again sectioned into 1-mm thick slices and incubated in 0.1% nitroblue tetrazolium in phosphate buffer at pH 7.4 and 37°C for 15 min. The tetrazolium dye forms a blue formazan complex in the presence of coenzyme and dehydrogenases. The necrotic portion of the myocardium at risk that did not stain was separated from the stained portion of the myocardium (that is, the ischemic but nonnecrotic area). All three portions of the left ventricular myocardium (nonischemic, ischemic nonnecrotic and ischemic necrotic) were weighed and the results are expressed as a percent of the total left ventricular mass; the area of necrotic tissue is computed as a percent of the area at risk and the total left ventricular mass. The three portions of the myocardium were then stored at -70°C for subsequent myeloperoxidase assay.

**Isolated coronary ring studies.** Both left circumflex and left anterior descending coronary artery segments removed were placed into warmed Krebs-Henseleit buffer consisting of (in mM): sodium chloride 118; potassium chloride 4.7; calcium chloride 2H₂O 2.54; potassium biphosphate 1.19; magnesium sulfate 7H₂O 1.19; sodium bicarbonate 125; and glucose 10. Isolated coronary vessels were cleaned and cut into rings of 2 to 3 mm in length. The rings were then mounted on stainless steel hooks, suspended in tissue baths and subsequently connected to FT-03 force displacement transducers (Grass Instrument) to record changes in tension on a Grass model 7 oscillographic recorder. The baths were filled with 20 ml of Krebs-Henseleit buffer and aerated at
37°C with a gas mixture of 95% oxygen and 5% carbon dioxide. Coronary rings were initially stretched to give a preload of 0.5 g of force, and allowed to equilibrate for 1 h. In a previous study, we have observed that preloads of ≥1 g caused injury to the endothelium and thus could not be used (15). During this period, the Krebs-Henseleit buffer in the tissue baths was replaced every 20 min. After equilibration, the rings were exposed to 10 ng/ml U-46619 (Upjohn), a thromboxane A2 mimic used to generate about 0.5 g of developed force. Once a stable contraction was obtained, 0.1, 1, 10 and 100 nM acetylcholine was added to the bath. After the response stabilized, the rings were washed and allowed to equilibrate to baseline once again. The procedure was repeated with ADP (0.001, 0.01, 0.1 and 1 µM) and then with sodium nitrite (0.1, 1, 10 and 100 µM). Sodium nitrite was prepared by dissolving the compound in 0.1 N hydrochloric acid and titrating it to pH 2. Titrating distilled water to pH 2 and adding aliquots to buffer in the bath did not produce any vasorelaxation.

**Determination of tissue myeloperoxidase activity.** Myocardial myeloperoxidase activity occurring virtually exclusively in neutrophils, was determined with the method of Bradley et al. (20) and Mullane et al. (21). The myocardium was homogenized in 0.5% hexadecyltrimethyl ammonium bromide (HTAB) (Sigma Chemical) and dissolved in 50 mM potassium phosphate buffer (pH 6) using a Potylron (PCU-2) homogenizer. Homogenates were centrifuged at 12,500 x g and 2°C for 30 min. The supernatants were then collected and reacted with 0.167 mg/ml of o-dianisidine dihydrochloride (Sigma Chemical) and 0.01105% hydrogen peroxide in 50 mM phosphate buffer (pH 6). The change in absorbance was measured spectrophotometrically at 460 nm. One unit of myeloperoxidase is defined as that quantity of enzyme hydrolyzing 1 µmol of peroxide/min at 25°C.

**Statistical analysis.** All values in the text, table and figures are presented as mean values ± SEM of n independent experiments. All data were subjected to analysis of variance followed by the Bonferroni correction for multiple comparisons by post-hoc t test. Probabilities <0.05 were considered to be statistically significant.

**Results**

**Hemodynamic and ECG changes.** In preliminary studies, we observed that there were no significant changes in any of the hemodynamic or biochemical variables observed during the 6-h observation period in cats with sham myocardial ischemia. Infusion of suprostone (60 ng/kg per min), human superoxide dismutase (0.25 mg/kg per h), or their combination had no effect on any of the variables observed in these cats (Table 1). Before occlusion of the left anterior descending coronary artery, heart rate, mean arterial blood pressure and pressure-rate index values were quite similar in the four groups of cats with myocardial ischemia. After occlusion, mean arterial blood pressure dropped sharply and heart rate decreased, leading to a sharp decline in pressure-rate index.

There was no significant difference among the four groups in mean arterial blood pressure, heart rate and pressure-rate index either during the ischemic period or after reperfusion. Therefore, any cardioprotective effect observed in any of the treated groups could not be attributed to a reduction in myocardial oxygen demand (Fig. 1).

No ST segment elevation was observed in any group of cats studied before coronary occlusion. However, soon after occlusion, the ST segment became significantly elevated, reaching a peak at 20 to 40 min after occlusion. Furthermore, ST segment elevations were not significantly different among any of the four groups studied, indicating that there were no significant differences in the severity of the ischemia produced as a result of the occlusion (Fig. 2). In cats in the sham occlusion group, ST segment elevations were not observed.
myocardial ischemia group, the ST segment was 0 ± 0 mV at all times.

Myocardial protection by human superoxide dismutase and taprostene. Plasma CK activity. Figure 3 summarizes the changes in plasma CK activity in all four groups of cats with myocardial ischemia. In preliminary studies in control sham myocardial ischemia cats, the plasma CK activity increased only slightly throughout the experiment, reaching a final value of 10 ± 3 IU/mg protein \( \times 10^{-3} \) and administration of human superoxide dismutase, taprostene, or their combination did not change the plasma CK activity (Table 1). However, large increases in plasma CK activity were observed in untreated cats subjected to myocardial ischemia and reperfusion. Neither the myocardial ischemia + taprostene group nor the myocardial ischemia + human superoxide dismutase groups showed significant protection when compared with the untreated myocardial ischemia group, because all cats given either agent alone exhibited markedly elevated CK activity associated with myocardial ischemia and reperfusion. There was no significant difference in plasma CK activity at any time point observed between taprostene- or human superoxide dismutase-treated groups and the untreated group.

In contrast, the myocardial ischemia + combined treatment group experienced a markedly lower plasma CK activity. The plasma CK activities of the myocardial ischemia + combined treatment group were significantly different from those of the myocardial ischemia + vehicle group at each time point after 2 h. Thus, combined treatment with low doses of taprostene and human superoxide dismutase effectively inhibited plasma accumulation of CK activity, an index of myocardial cellular injury.

Percent necrotic tissue. Another more direct index of myocardial injury induced by ischemia and reperfusion is anatomic estimation of necrotic tissue. The wet weights of the areas of myocardium subjected to ischemia (area at risk) expressed as a percent of the total left ventricular weights were not significantly different among any groups, providing additional evidence that the severity of the ischemic insult was comparable in all myocardial ischemia groups. In contrast, the weights of the necrotic myocardial tissue expressed either as a percent of the area at risk or as the total left ventricular weight were not equivalent among these groups. A relatively large percent of necrotic tissue was observed in the untreated myocardial ischemia group. Treatment with either low dose taprostene or human superoxide dismutase alone only slightly decreased the percent of necrotic myocardial tissue. There were no statistically significant differences among these three groups. However, when low doses of taprostene and human superoxide dismutase were infused together, the percent of necrotic myocardial tissue expressed either as area at risk or as total left ventricular mass was significantly lower than in any of the other myocardial ischemia groups (Fig. 4), indicating a significant cardioprotective effect of the combined therapy. Area at risk was 0 in all cats with sham myocardial ischemia, in four vehicle-treated cats, the area at risk was randomly divided into two portions and incubated with microtubule tetrazolium solution separately in the presence and absence of 20 ng/ml human superoxide dismutase and 10 ng/ml taprostene. The results indicated that addition of human superoxide dismutase and taprostene in vitro did not have any effect on mucrobile tetrazolium staining.

Effect of human superoxide dismutase and taprostene on changes in myocardial myeloperoxidase activity. One of the major mechanisms thought to be responsible for myocardial reperfusion injury is infiltration of neutrophils into the reperfused ischemic region. Myeloperoxidase activity is specific for neutrophils, and generally accepted as a marker for

![Figure 3. Plasma creatine kinase (CK) activity expressed as international units (IU)/mg protein \( \times 10^{-3} \) measured at various times during 1.5 h of ischemia and after 4.5 h of reperfusion. All values are mean values ± SEM of five to seven cats. Plasma CK activity rises slightly following ischemia and increases significantly after reperfusion. □ —■ = myocardial ischemia + vehicle; ■ —■ = myocardial ischemia + human superoxide dismutase; other symbols as in Figure 1.]

Table 1. Effects of Human Superoxide Dismutase and Taprostene on Hemodynamic Variables and Creatine Kinase Activity in Cats With Sham Myocardial Ischemia

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<tr>
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<th>HR (beats/min)</th>
<th>MAPB (mm Hg)</th>
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<th>CK (IU/mg protein ( \times 10^{-3} ))</th>
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Values are mean values ± SEM for four to six cats. CK = creatine kinase; HR = heart rate; HSOD = human superoxide dismutase (0.25 mg/kg per h); MAPB = mean arterial blood pressure; PRI = pressure rate index; Tap = taprostene (50 ng/kg per min).
neutrophil accumulation in the heart. Figure 5 illustrates myeloperoxidase activities in heart tissue samples from the four myocardial ischemia groups. In the nonischemic non-reperfused myocardium (that is, area not at risk), myeloperoxidase activity was very low in all groups and there were no significant differences among any of the groups. However, marked increases in myeloperoxidase activity were observed in the area at risk and the necrotic area in the myocardial ischemia + vehicle group. When taprostene or human superoxide dismutase was infused alone, cardiac myeloperoxidase activity remained markedly elevated. In contrast, the combined treatment group exhibited markedly lower cardiac myeloperoxidase activities in both the area at risk and the necrotic area, indicating that the combination of low dose taprostene with low dose human superoxide dismutase significantly inhibits neutrophil infiltration into the ischemic myocardium. Cardiac myeloperoxidase activity in cats with sham myocardial ischemia was $0.04 \pm 0.02$ U/100 mg tissue.

**Figure 6.** Representative recording of endothelium-dependent vasodilators, acetylcholine (ACh) and A23187, and endothelium-independent vasodilator, sodium nitrate (NaNO$_2$) induced relaxation of precontracted (U-46619) ischemic and reperfused left anterior descending (LAD) coronary artery rings. The arrows indicate the addition of U-46619; the dots indicate the addition of acetylcholine or sodium nitrite. Acetylcholine and A23187 induced almost no relaxation to rings from cats in the myocardial ischemia (MI) + vehicle group. Neither taprostene nor human superoxide dismutase (SOD) administered alone improved the response of coronary rings to acetylcholine. However, the acetylcholine and A23187 responses of rings isolated from a cat with myocardial ischemia given combined treatment with taprostene and human superoxide dismutase were significantly preserved.

Figure 6 illustrates typical recordings of ischemic left anterior descending coronary rings obtained from the different groups. The response of coronary rings obtained from the myocardial ischemia + vehicle group to the endothelium-dependent vasodilators, acetylcholine and A-23187, was almost totally abolished at 4.5 h after reperfusion, and neither taprostene nor human superoxide dismutase infusion alone preserved the vasorelaxant responses to either acetylcholine or A-23187. However, these coronary rings relaxed...
fully when the endothelium-independent vasodilator sodium nitrite was added, indicating that the responsiveness of the coronary vascular smooth muscle to direct vasodilators remained normal. In contrast, rings obtained from the combined taprostene- and human superoxide dismutase-treated group significantly relaxed in response to both endathelium-dependent vasodilators (acetylcholine and A-23187) and to the endothelium-independent vasodilator (sodium nitrite). Figure 7 summarizes the vasorelaxant responses to acetylcholine, A-23187 and sodium nitrite in isolated cat left anterior descending coronary artery rings. Clearly, the response of ischemic rings to the endothelium-dependent vasodilators was significantly preserved by combined taprostene and human superoxide dismutase treatment. Vasorelaxant responses to acetylcholine and A-23187 were 96 ± 6% and 97 ± 5%, respectively, in left anterior descending coronary artery rings isolated from cats with sham myocardial ischemia. Moreover, the nonischemic left circumflex rings from all groups showed equal and complete relaxation in response to acetylcholine, A-23187 and sodium nitrite. There were no significant differences in response to any of the vasodilators studied among the groups of left circumflex coronary artery rings. Thus, nonischemic coronary artery rings relaxed completely in response to all vasodilators, indicating normal endothelium and vascular smooth muscle.

Discussion

Role of free radicals in reperfusion injury. The early restoration of myocardial blood flow after myocardial ischemia is essential to retard the progression of myocardial cell death and to permit the functional recovery of reversibly injured myocardium. However, many studies also point to a potentially detrimental effect of reperfusion on endothelial integrity and myocardial function (12,22). Although the cause of this reperfusion-induced enhancement of cardiac injury is apparently multifactorial, a mounting body of evidence now indicates that oxygen-derived free radicals are important mediators of ischemia-reperfusion-induced endothelial and myocardial injury (2,15,23,24).

A wide variety of cells, organelles and enzymes may be involved in the free radical formation activated by ischemia and reperfusion. These include neutrophils, xanthine oxidase, cyclooxygenase and lipoxygenase, autoxidation of catecholamines, mitochondria and the sarcoplasmic reticulum (10,25,26). Among these, xanthine oxidase localized in endothelial cells (27-29) and nicotine adenine dinucleotide phosphate, reduced form (NADPH) found in neutrophils (30) have been demonstrated to be important sources of free radicals in the reperfused ischemic heart. Free radicals produced by xanthine oxidase in endothelial cells could act as a powerful chemotactic signal for neutrophils. Neutrophils would then adhere to endothelial cells and generate larger amounts of free radicals resulting in endothelial and myocardial injury. This sequence has been termed an "endothelial cell trigger" and "neutrophil amplifier" mechanism by Bulkey (30).

Oxygen-derived free radicals may exert diverse biochemical effects on both intracellular and extracellular sites, and there is extensive evidence that cardiac structure and function can be altered by these effects (25). It has been demonstrated that free radicals can initiate lipid peroxidation, alter membrane permeability to ions and inactivate endothelium-derived relaxing factor (16), which is a potent promoter of endothelial and cardiac muscle cells in ischemia and reperfusion (21). Further evidence for the role of free radicals as a major mediator of reperfusion injury was derived from the studies in which free radical scavengers limited ultimate infarct size (13) and preserved the endothelium-dependent relaxation of the reperfused ischemic coronary artery (15,22). Infusion of higher doses of human superoxide dismutase can scavenge the superoxide radicals produced from xanthine oxidase, thereby decreasing neutrophil recruitment (32). Human superoxide dismutase can also scavenge the superoxide produced by neutrophils or any other cells and therefore can protect endothelial cells and myocardium from reperfusion injury directly.

Role of neutrophils in reperfusion injury. On the other hand, substances that inhibit neutrophil adherence and activation, like monoclonal antibodies against CD11/CD18 adherence glycoprotein of neutrophils (6) and prostacyclin (33), have been shown to be effective in protecting against endothelial and myocardial injury associated with ischemia and reperfusion. Prostacyclin is a synthetic, chemically stable analogue of prostaglandin that has been specifically designed to enhance its cytoprotective actions while minimizing unwanted hemodynamic effects (34). In a previous study (9), we observed that infusion of taprostene at a rate of 100 ng/kg per min exerted significant endothelial and cardioprotective effects in a cat model of myocardial ischemia and reperfu-
There are a variety of possible mechanisms by which taprostene could afford cardioprotection. Because infusion of taprostene at a rate of 100 ng/kg per min does not induce any significant changes in mean arterial blood pressure or pressure-rate index, it is unlikely that taprostene produces a significant coronary steal. However, taprostene significantly attenuated myeloperoxidase activity, a marker of neutrophil infiltration, both in the area at risk and in the necrotic area, implying that prevention of neutrophil adherence and activation is a primary mechanism by which taprostene provides its endothelial and cardioprotection.

Although a higher dose of human superoxide dismutase exerts significant cardioprotective effect in some experiments, the relatively short pharmacologic half-life (6 to 10 min) of this agent presents a drawback to its study in experimental protocols extending for several days after reperfusion. Moreover, a recent clinical investigation (7) indicated that administration of human superoxide dismutase had no protective effect against myocardial injury in humans. Neutrophil chemotaxis to the ischemically injured myocardium and the local formation of neutrophil-derived free radicals continue to exert a cytotoxic influence on the viable myocardial cells in the reperfused tissue. Therefore, it would be expected that using substances that inhibit neutrophil adherence and activation together with human superoxide dismutase should have additive effects in protecting endothelial cells and myocardium from reperfusion injury.

Beneficial effect of combined human superoxide dismutase and taprostene treatment on reperfusion injury. We obtained several lines of evidence that the combination of taprostene and human superoxide dismutase exerts a cardioprotective effect in ischemia and reperfusion. First, plasma CK activities, indicative of the severity of ischemic injury to the myocardium, were significantly lower in cats treated with taprostene and human superoxide dismutase together than in cats receiving taprostene or human superoxide dismutase alone or their vehicle. Second, although analysis of myocardial tissue clearly indicated that all four ischemic groups were exposed to a comparable degree of myocardial jeopardy as a result of left anterior descending coronary artery occlusion, a relatively large proportion of the area at risk became necrotic in untreated cats with myocardial ischemia and in cats given taprostene or human superoxide dismutase alone. Infusion of taprostene and human superoxide dismutase together prevented much of the ischemic tissue from becoming necrotic, whereas whether as a percent of the area at risk or as a percent of the total left ventricular mass. Third, the coronary ring data indicate that ischemia and reperfusion produced endothelial dysfunction characterized by a reduced vasorelaxing response to the endothelium-dependent dilators, acetylcholine and A-23187. In contrast, coronary rings from cats with myocardial ischemia and combination treatment exhibited a significantly greater vasorelaxant response to acetylcholine and A-23187, indicating that endothelial function as exemplified by producing and releasing endothelium-derived relaxing factor was preserved by combined treatment with taprostene and human superoxide dismutase. Similar findings occur in the ischemic reperfused isolated rat heart, indicating that the microvascular endothelium responds similarly to that of large coronary arteries (29).

Neither human superoxide dismutase alone nor taprostene alone in the low dose used in this study sufficiently inhibited neutrophil adherence or infiltration. However, when these two substances were given together, a significantly decreased myeloperoxidase activity was observed, in both the area at risk and the necrotic area. These results suggest that administration of low dose human superoxide dismutase can only partly scavenge superoxide radicals, which either act as a powerful chemotactic factor or allow the expression of chemotactic factors for neutrophil adherence and activation. However, administration of low dose taprostene can partly inhibit neutrophil adherence. When the two substances were used together, an enhanced effect was obtained. Therefore, neutrophil adherence and infiltration were prevented and the endothelial and myocardial injury that was presumably contributed to by activated neutrophils was significantly decreased.

Conclusions. In summary, occlusion of the left anterior descending coronary artery in cats for 90 min followed by 4.5 h of reperfusion resulted in significant endothelial dysfunction and myocardial injury. The doses of human superoxide dismutase and taprostene employed in this study were lower than those that produced significant endothelial and myocardial protection when the agents were administered separately in this model of myocardial ischemia and reperfusion. However, when these agents were combined, highly significant beneficial effects occurred, indicating an enhanced effect of these two substances 6 h after the onset of myocardial ischemia. Although these results do not indicate that such a protective effect occurs at 48 h after ischemia, when the infarction process is complete, it represents a significant early anti-ischemic effect. Further work on a 48-h model of ischemia and reperfusion would be helpful in assessing the clinical usefulness of these findings.

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SUPEROXIDE DISMUTASE AND PROSTACYCLIN ANALOGUE IN ISCHEMIA
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