BRIEF COMMUNICATIONS

The Independent Effects of Oxygen Radical Scavengers on Canine Infarct Size
Reduction by Superoxide Dismutase but not Catalase

Steven W. Werns, Michael J. Shea, Edward M. Driscoll, Christopher Cohen, Gerald D. Abrams, Bertram Pitt, and Benedict R. Lucchesi

From The University of Michigan Medical School, Departments of Pharmacology, Pathology, and Internal Medicine (Division of Cardiology) Ann Arbor, Michigan

SUMMARY. Previous studies demonstrated a significant reduction of ultimate infarct size in the canine heart by the combined administration of superoxide dismutase plus catalase. This study was performed to assess the independent effects of each enzyme on ultimate infarct size due to ischemia/reperfusion. Dogs received 2-hour infusions of superoxide dismutase, catalase, or albumin (controls) via the left atrium beginning 15 minutes before and ending 15 minutes after a 90-minute occlusion of the left circumflex coronary artery. The dogs were killed 6 hours after reperfusion. After histochemical staining, infarct and risk area masses were calculated by gravimetric and planimetric analysis. Infarct size expressed as a percentage of the area at risk was: superoxide dismutase, 19 ± 5; catalase, 30 ± 5; and controls, 40 ± 3. Infarct size in the superoxide dismutase group, but not the catalase group, was significantly less than in controls (P < 0.05). No significant differences in hemodynamics or area at risk were observed that could explain the differences in infarct size. The results indicate that superoxide dismutase alone protects reperfused ischemic myocardium as well as does the combination of superoxide dismutase and catalase. The beneficial effect of superoxide dismutase and insignificant effect of catalase suggest that tissue damage during ischemia and reperfusion may be mediated largely by superoxide anion but not by hydrogen peroxide. (Circ Res 56: 895-898, 1985)

TISSUE injury resulting from a variety of causes is attributable to the toxic effects upon biological systems of reactive oxygen species, such as superoxide anion (-O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radical (-OH) (Freeman and Crapo, 1982). Studies of intestinal (Parks et al., 1982), cerebral (Demopoulos et al., 1980), and global cardiac (Shlafer et al., 1982; Gardner et al., 1983) ischemia implicate oxygen-free radicals in the development of ischemic tissue injury.

Previous experiments reported by this laboratory (Jolly et al., 1984) investigated the effects upon regional myocardial ischemia of the combined administration of superoxide dismutase (superoxide oxidoreductase EC 1.15.1.1) and catalase (H₂O₂:H₂O oxidoreductase EC 1.11.1.6), enzymes which are endogenous scavengers of -O₂⁻ and H₂O₂, respectively. In a canine model of myocardial infarction, the combination achieved a 50% reduction of infarct size assessed 24 hours after a 90-minute circumflex coronary artery (LCX) occlusion followed by reperfusion. Although the results suggest that myocardial necrosis due to coronary occlusion and reperfusion may be limited by free radical scavengers, the relative importance of -O₂⁻ and H₂O₂ in the pathophysiological process could not be deduced. Therefore, this study was performed to determine the effect of either enzyme on the extent of myocardial injury after ischemia and reperfusion.

Methods

Detailed methods have been published previously (Romson et al., 1983). Briefly, male mongrel dogs (12-15 kg) were anesthetized with Dial-urethane (0.6 ml/kg, iv) and ventilated with room air. After thoracotomy, the proximal LCX was affixed with an electromagnetic flow probe and a critical stenosis of silk suture. Left atrial pressure, carotid artery pressure, LCX blood flow, and lead II ECG were recorded.

The treatment groups were: (1) bovine erythrocyte superoxide dismutase (SOD), 5 mg/kg (3,800 U/mg, Boehringer, or 3,000 U/mg, Sigma); (2) bovine liver catalase (CAT), 5 mg/kg (11,000 U/mg, Sigma); and (3) bovine serum albumin fraction V, 15 mg/kg (Calbiochem), dissolved in 60 ml of 0.9% sodium chloride. Albumin was selected as a nonspecific protein control for any osmotic effects of the enzymes. The solutions were infused via the left atrium at 0.5 ml/min starting 15 minutes before and ending 15 minutes after a 90-minute LCX occlusion. Reperfusion was initiated gradually over 30 minutes.

Six hours after onset of reperfusion, the heart was fibrillated electrically and excised. Using a dual perfusion technique previously described (Shea et al., 1984) followed by fixation in 10% formalin, infarct and risk area masses were calculated by both gravimetric and planimetric analysis.
Samples of triphenyltetrazolium chloride (TTC)-negative tissue along with the adjacent TTC-positive border were coded, fixed in 10% buffered formalin (pH 7.4, 37°C), embedded in paraffin, cut into 5-μm-thick sections, and stained with hematoxylin and eosin. A cardiac pathologist (G.D.A.), unaware of the treatment protocol, graded the samples on a semiquantitative scale for microscopic evidence of necrosis and leukocyte infiltration.

All data were expressed as mean ± SEM. Paired t-tests were used to analyze group differences in coronary blood flow (Armitage, 1971). Group comparisons of hemodynamic data were made by analysis of variance (Armitage, 1971) and Duncan’s multiple range test (Duncan, 1955). Group comparisons of infarct data were made by analysis of variance (Armitage, 1971) and Scheffe’s test (Neter and Wasserman, 1974). Differences were considered significant when P < 0.05.

Results

Dogs were excluded from analysis for heartworms or failure to develop objective evidence of regional ischemia upon LCX occlusion (no S-T segment change or epicardial cyanosis). Dogs were also excluded if more than two cardioversions were required to reverse ventricular fibrillation, or if a fatal arrhythmia occurred before 4 hours of reperfusion (4 CAT, 3 SOD, 1 control). One experiment (control) was terminated early because of a failure to develop albumin or enzyme infusion preceding LCX occlusion. There were also no significant intergroup differences in these parameters before, during, or after LCX occlusion (Table 1). The mean left atrial pressure rose 15 minutes after LCX occlusion by 3.3 mm Hg in controls, 3.1 mm Hg in the SOD group, and 3.0 mm Hg in the CAT group. For each group, the mean LCX flow 6 hours after reperfusion was significantly lower than preocclusion, but there were no differences among the groups (Table 1).

Reduction of Myocardial Infarct Size

Body weight, left ventricular mass, and area at risk as a percent of left ventricle for each group were not significantly different. By both gravimetric and planimetric analysis, infarct size in the SOD group was significantly smaller than controls (P < 0.05, Fig. 1). SOD reduced infarct size by greater than 50%, whether infarct mass was expressed as a percentage of the left ventricle or area at risk. Infarct size in the CAT group, however, was not significantly different from controls (P > 0.05). As demonstrated in previous studies from this laboratory (Jolly and Lucchesi, 1983), gravimetric and planimetric analysis of IZ/LV, IZ/AR, and AR/LV (IZ = infarct zone, LV = left ventricle, AR = area at risk) were in close agreement. The planimetric (P) and gravimetric (G) determinations of IZ/AR were related as follows: P = 0.89 (G) + 1.29, r = 0.91, P < 0.001, see 6.52, n = 30.

Hemodynamic Parameters

Heart rate, mean arterial pressure, rate-pressure product, left atrial pressure, and LCX blood flow did not change significantly during the initial 15 minutes of albumin or enzyme infusion preceding LCX occlusion. There were also no significant intergroup differences in these parameters before, during, or after LCX occlusion (Table 1). The mean left atrial pressure rose 15 minutes after LCX occlusion by 3.3 mm Hg in controls, 3.1 mm Hg in the SOD group, and 3.0 mm Hg in the CAT group. For each group, the mean LCX flow 6 hours after reperfusion was significantly lower than preocclusion, but there were no differences among the groups (Table 1).

Histological Analysis of Risk Region

Histological examination of the border between the TTC-positive and -negative tissue confirmed the presence within the TTC-negative region of histo-

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Hemodynamic Data</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time</strong></td>
<td><strong>Heart rate (beats/min)</strong></td>
</tr>
<tr>
<td>Control</td>
<td>134 ± 22</td>
</tr>
<tr>
<td>Preocclusion</td>
<td>131 ± 25</td>
</tr>
<tr>
<td>End occlusion</td>
<td>178 ± 38</td>
</tr>
<tr>
<td>End reperfusion</td>
<td>134 ± 33</td>
</tr>
<tr>
<td>CAT</td>
<td>129 ± 20</td>
</tr>
<tr>
<td>Preocclusion</td>
<td>181 ± 49</td>
</tr>
<tr>
<td>End occlusion</td>
<td>136 ± 22</td>
</tr>
<tr>
<td>End reperfusion</td>
<td>132 ± 18</td>
</tr>
<tr>
<td>SOD</td>
<td>155 ± 22</td>
</tr>
</tbody>
</table>

* Mean ± SEM.
† P < 0.05 vs. preocclusion.
logical signs of necrosis. The degree of leukocyte infiltration of infarcted tissue was quite variable, with no discernible difference between groups.

**Discussion**

This study demonstrates that a 2-hour infusion of superoxide anion scavenger, SOD, reduces by 50% the extent of myocardial injury after regional ischemia and reperfusion of 90 minutes and 6 hours, respectively. Using the same protocol and dose (5 mg/kg), a scavenger of hydrogen peroxide, catalase, had no significant effect. For the sample size of 10, the probabilities (type II error) of not detecting 50% or 40% reductions of infarct mass are approximately 15% or 30%.

In a previous study using the same duration of ischemia but longer reperfusion (24 hours), the same doses of SOD and CAT in combination also reduced infarct size by 50% (Jolly et al., 1984). Therefore, for the doses used, no additive or synergistic effects of the combination are apparent, although a larger dose of catalase might have a greater effect than the dose used in this study.

In vitro studies suggest that cellular damage, e.g., peroxidation of lipids by H\textsubscript{2}O\textsubscript{2} and \textbullet{O}2\textsuperscript{−}, may be mediated by more toxic daughter species. Ferrous iron can catalyze reduction of H\textsubscript{2}O\textsubscript{2} to hydroxyl radical, and neutrophil myeloperoxidase can catalyze the reaction of H\textsubscript{2}O\textsubscript{2} with chloride to form hypochlorous acid (Fantone and Ward, 1982). The low pH of ischemic myocardium favors conversion of \textbullet{O}2\textsuperscript{−} to the more reactive perhydroxyl radical (\textbullet{HO}_{2}\textsuperscript{−}) (Freeman and Crapo, 1982). Since SOD should increase tissue H\textsubscript{2}O\textsubscript{2} and CAT should decrease it, this study suggests that adequate endogenous pathways of H\textsubscript{2}O\textsubscript{2} detoxification persist after ischemia, or that \textbullet{HO}_{2}\textsuperscript{−} is the predominant oxygen radical formed within ischemic myocardium.

Since SOD did not reduce myocardial oxygen demand, the inflammatory aspects of myocardial ischemia and anti-inflammatory properties of SOD merit consideration. Myocardial ischemia elicits an inflammatory response that includes activation of leukocytes (Pinckard et al., 1979, 1980) and kinin (Torstila, 1978), release of chemotactic factor (Hartmann et al., 1977), migration of neutrophils (Mullan et al., 1984), and peroxidation of lipids (Rao and Mueller, 1983). Suppression of neutrophil infiltration of ischemic myocardium by anti-inflammatory drugs like ibuprofen (Romson et al., 1982) and nafazatrom (Shea et al., 1984), and granulocytopenic agents such as hydroxyurea (Mullan et al., 1984) and neutrophil antiserum (Romson et al., 1983), appears to reduce ischemic injury. SOD attenuates immunologically induced neutrophil accumulation, edema formation, and tissue destruction, possibly by suppressing generation of chemotactic lipids by \textbullet{O}2\textsuperscript{−} (Fantone and Ward, 1982). Therefore, SOD may curtail injury to reperfused ischemic myocardium by preventing the inflammatory response to ischemia.

The growing recognition that oxygen radicals play a role in ischemic myocardial injury mandates reexamination of previously reported studies. For example, allopurinol's antiischemic characteristics have been ascribed to preservation of a purine pool for ATP synthesis (DeWall et al., 1971), but may reflect diminished \textbullet{O}2\textsuperscript{−} generation by inhibition of xanthine oxidase (Gardner et al., 1983). Limitation of infarct size by mannitol was considered an osmolar effect on cell swelling, but quenching of hydroxyl radical may be the true mode of action (Magain et al., 1984). The disparate effects on infarct size of cyclooxygenase inhibitors may be a function of their differential abilities to inhibit \textbullet{O}2\textsuperscript{−} release by neutrophils. For example, aspirin does not reduce infarct size or \textbullet{O}2\textsuperscript{−} production by neutrophils, but ibuprofen decreases both (Flynn et al., 1984). Prostaglandin's suppressive influence on oxygen radical release by neutrophils (Fantone and Kinnes, 1983) may explain its limitation of infarct size by nonheme-dynamic means (Melin and Becker, 1983).

Although animal data must be applied to human disease with caution, oxygen radical-mediated damage may be facilitated by current therapeutic approaches to acute myocardial infarction. Reperfusion of ischemic myocardium by streptokinase may increase the oxygen available to generate free radicals (Gauduel and Duvelleroy, 1984) and promote the formation of chemotactic factors from fibrin, complement, and kinin (Chesterman, 1978). An oxygen radical scavenger such as SOD, which has been applied to other inflammatory conditions, (Huber and Menander-Huber, 1980), might increase the amount of ischemic tissue salvaged by myocardial reperfusion and thereby improve the prognosis of patients with myocardial infarction.
The free radical pathology and the microcirculation in the major central nervous system disorders. Acta Physiol Scand 492: 91–119


Duncan DB (1955) Multiple range and multiple F-tests. Biometrics 11: 1–42


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