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Anti-Shock Effects of Human Superoxide Dismutase in Splanchnic Artery Occlusion (SAO) Shock (42734)

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Abstract. We studied the effects of human superoxide dismutase (h-SOD) in splanchnic artery occlusion (SAO) shock. Pentobarbital anesthetized rats subjected to total occlusion of the superior mesenteric and the celiac arteries for 40 min developed a severe shock state usually resulting in a fatal outcome within 20 min after the release of the occlusion. h-SOD (10 mg/kg) was infused intravenously starting at reperfusion and lasting for 10 min. SAO shock rats treated with h-SOD maintained postreperfusion MABP at significantly higher values compared to rats receiving the vehicle (final MABP 84 ± 6 vs 46 ± 1 mm Hg, $P < 0.01$, respectively). Treatment with h-SOD attenuated the plasma accumulation of free amino-nitrogen compounds ($P < 0.01$ from vehicle) as well as the activity of the lysosomal protease cathepsin D ($P < 0.05$ from vehicle). Furthermore, the plasma activity of a myocardial depressant factor was significantly lower in h-SOD-treated rats than in SAO rats receiving only the vehicle (27 ± 1 vs 64 ± 3 U/ml, $P < 0.01$). SAO shock rats treated with h-SOD also exhibited a significantly higher survival rate than the SAO shock ± vehicle group (88% vs 11%, $P < 0.01$, respectively). These results support the role of oxygen-derived radicals in the pathophysiology of SAO shock, and indicate that h-SOD effectively ameliorates the deleterious effects of oxygen radicals in this severe model of ischemia and reperfusion.

Splanchnic artery occlusion (SAO) shock is a severe form of circulatory shock produced by ischemia of the splanchnic organs. This type of shock is characterized by a marked decrease in systemic blood pressure after reperfusion of the splanchnic circulation, usually leading to a fatal outcome (1). Other important characteristics of SAO shock are intestinal mucosal lesions, increased microvascular permeability leading to fluid loss into the intestinal lumen, local splanchnic release of lysosomal hydrolases, enhanced proteolysis, and accumulation of a myocardial depressant factor (MDF) in the blood (1–3).

Oxygen-free radicals have recently been shown to play a major role in the pathophysiology of injuries caused by reperfusion of ischemic tissues in a variety of organs and animal species (4–7). In the splanchnic region, the oxygen radicals have been shown to be mediators of ischemic damage (8) to the small bowel. Furthermore, a growing body of evidence also implicates oxygen-free radicals in the pathophysiology of acute pancreatitis (9) and in ischemia–reperfusion injury to the liver (10). Although the precise mechanisms of action of oxygen-derived radicals in this regard has not yet been fully elucidated, it appears that the superoxide anion (O_2) is important in the pathogenesis of splanchnic ischemia and that inhibition of its synthesis (e.g., by allopurinol) or enhancement of its degradation by superoxide dismutase (SOD) exerts protective effects on microvascular and parenchymal damage induced by ischemia and reperfusion in the splanchnic region (8).

The purpose of the present study was to evaluate the effects of human recombinant superoxide dismutase (h-SOD) (11) in a severe model of SAO shock and reperfusion, and to assess its effects on important metabolic and cellular consequences of SAO shock including plasma proteolysis and the plasma accumulation of lysosomal hydrolase and MDF activities in the rat.

Materials and Methods. Experiments were performed on male Sprague–Dawley rats weighing 240–280 g. The rats were anesthetized with sodium pentobarbital (40 mg/kg) administered intraperitoneally. All

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animals were subjected to the same surgical procedure. The trachea was exposed and cannulated to ensure an open airway. A polyethylene catheter (PE-50) was placed in the right carotid artery for recording of mean arterial blood pressure (MABP) and heart rate (HR). MABP was recorded on a Grass Model 7 oscillographic recorder using Statham P23AC pressure transducers. A polyethylene catheter (PE-50) was inserted into the left external jugular vein for drug or vehicle infusion.

Esophageal temperature was monitored and maintained at 36 ± 1°C throughout the experiment using a Yellow Springs Model 73-A temperature controlling unit. After a midline laparotomy, the celiac and the superior mesenteric arteries were isolated near their aortic origins. After completion of surgery, the animals were given heparin (500 U/kg, iv) and were observed for a 30-min stabilization period prior to the start of the experiment.

Splanchnic artery occlusion (SAO) shock was induced by totally occluding the celiac artery and the superior mesenteric artery for 40 min. Following this period of splanchnic ischemia, the occlusive clamps were removed and the animals were observed for an additional 120 min or until MABP fell below 45 mm Hg. Rats failing to maintain a MABP of 45 mm Hg for 30 min postrelease were excluded from the study. One SAO + vehicle rat was thus excluded from the study. Survivors are defined as rats maintaining a MABP of >45 mm Hg for 120 min postrelease. The sham shock rats were subjected to all surgical procedures employed with the SAO shock animals except that the splanchnic arteries were not clamped.

We infused h-SOD or its vehicle (i.e., 0.9% NaCl) over 10 min, concomitantly with the start of reperfusion in SAO shock rats, or at the same time as in sham shock rats. The h-SOD obtained from Grunenthal AG (Aachen, FRG) had an activity of 3100 SOD U/mg.

The animals were divided into three groups: (a) sham shock rats given h-SOD (10 mg/kg) (N = 6); (b) SAO shock rats given only the vehicle (0.9% NaCl, 0.35 ml) (N = 9); and (c) SAO shock rats given h-SOD (10 mg/kg) (N = 8). The dose of h-SOD employed (i.e., 10 mg/kg) was given to four sham SAO shock rats and no changes were observed either in the hemodynamic or in the biochemical variables measured. Blood samples (0.8 ml) were drawn at the end of the stabilization period and at the end of the experiment for analysis of plasma protein concentration, cathepsin D activity, and the hematocrit. Plasma-free amino-nitrogen concentration and plasma activities of MDF were determined at the end of the experiment. All blood volume removed by sampling was replaced by an equal amount of 0.9% NaCl.

Plasma analysis. Blood samples were kept on ice until they were centrifuged at 2500g and 4°C for 20 min. The plasma was removed and analyzed for protein concentration by the biuret method of Gornall *et al.* (12). The plasma activity of cathepsin D, a lysosomal hydrolase, was measured by the method of Anson (13) with bovine hemoglobin as a substrate, and indexed with plasma protein concentration. Plasma-free amino-nitrogen concentration was determined by the method of Kabat (14) using deproteinized plasma samples. Free amino-nitrogen was used as an index of total plasma proteolysis and is expressed in U/ml. One unit equals one micromole serine $\times 10^{-2}$. Myocardial depressant factor (MDF) activity was determined in deproteinized plasma using paper chromatography by the method of Barenholz *et al.* (15). The chromatographic spots corresponding to MDF were developed with ninhydrin and eluted in $NaHCO_3$ according to the method of Yamada and Pettit (16). The absorbance of each eluate was determined at 570 nm, and the value was related to the absorbance of the eluted serine standard. One MDF chromatographic unit is equal to the absorbance of 13 nmol of serine.

Statistical methods. All values in the text are means ± SEM. Differences among multigroup means were compared by analysis of variance (ANOVA). Tukey's pairwise comparison was used to determine the significance between specific pairs of data means. Analysis of survival was done by the χ^2 test.

Results. The model of SAO shock employed in this study is severe, and untreated shock animals exhibited marked postreinfusion hypotension accompanied by a high

mortality rate. Thus, eight of nine SAO + vehicle rats (i.e., 89%) exhibited a fall in MABP below 45 mm Hg within 120 min postrelease and thus are not considered as survivors. All groups of rats exhibited comparable initial MABP in the range of 120 to 130 mm Hg. Furthermore, MABP in the sham shock group did not change significantly throughout the experimental period indicating that the surgical procedure itself does not contribute significantly to the severity of the shock state.

The time course of MABP in the different experimental groups is illustrated in Fig. 1. After occlusion of the splanchnic arteries, all SAO shock groups exhibited a similar increase in MABP ranging from 20 to 25 mm Hg, followed by a decrease to values which were not significantly different from preocclusion control MABP values. Upon release of the occlusive clamps, the two groups of SAO rats experienced a rapid hypotensive phase to about 60 mm Hg, followed by an increase to a MABP of 91 to 95 mm Hg 20 min later (at time 60 min). SAO shock rats receiving the vehicle then exhibited a gradual secondary decrease in MABP reaching a final value of 45 ± 1 mm Hg. In contrast, SAO shock rats treated with h-SOD maintained MABP at values significantly higher than SAO shock rats receiving only the vehicle throughout the postreperfusion period. The final MABP of the h-SOD-treated SAO shock group was 84 ± 6 mm Hg ($P < 0.01$ from the SAO shock + vehicle group).

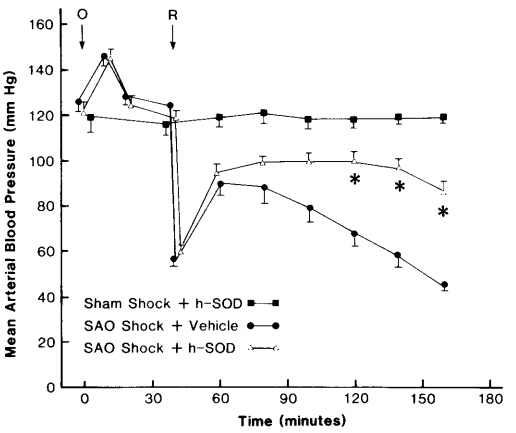

FIG. 1. Time course of mean arterial blood pressure in sham-shock and splanchnic artery occlusion (SAO) shock. All values are means ± SEM. The number of animals is six in the sham shock group, nine in the SAO + vehicle group, and eight in the SAO shock + h-SOD group. *SAO shock + h-SOD values significantly higher ($P < 0.01$) than SAO shock + vehicle. O, occlusion; R, release of occlusion.

Disruption of lysosomal membranes and leakage of lysosomal hydrolase activity into the blood is an important consequence of splanchnic ischemia. Plasma activities of the lysosomal hydrolase cathespin D at the beginning of the experiment were comparable among the three experimental groups and ranged between 4.0 and 5.0 U/mg protein. Figure 2 summarizes the plasma activities of cathepsin D at the end of the experiment for all the experimental groups. The activity of the sham shock group did not change significantly during the course of the experiment. In contrast, SAO shock rats receiving the vehicle exhibited cathepsin D activities which were sixfold greater than their initial value or the final value observed in sham shock rats. Treatment with h-SOD resulted in a moderate attenuation of the increase in plasma cathepsin D in shock rats ($P < 0.05$).

Increased plasma proteolysis, largely resulting from the action of lysosomal hydrolases, is also an important sequelae of splanchnic ischemia. Plasma amino-nitrogen concentration represents a marker of the degree of plasma proteolysis. Figure 3 illustrates plasma amino-nitrogen concentrations at the end of the experiment. Splanchnic artery occlusion shock increased free amino-nitrogen concentration in the SAO shock group receiving the vehicle to values over twofold greater than those of sham shock rats at the same time. However, treatment with h-SOD significantly diminished the increase in plasma-free amino-nitrogen when compared with the SAO shock + vehicle group ($P < 0.01$).

Generation of the cardiodepressant substance, MDF, is another important event in the pathophysiology of SAO shock resulting from enhanced proteolysis. Final plasma MDF activities are shown in Fig. 4. Sham rock rats receiving h-SOD exhibited low plasma MDF activities. However, splanchnic ischemia induced a threefold increase in plasma MDF activities in rats receiving the vehicle ($P < 0.01$). In contrast, h-SOD-

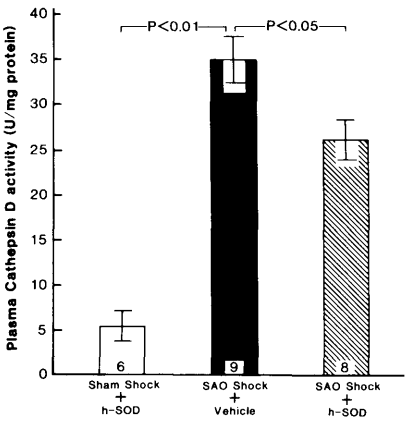

FIG. 2. Plasma cathepsin D activities at the end of the experiment in three experimental groups of sham shock and SAO shock rats. Bar heights are means; brackets indicate ±SEM. The numbers in the bars indicate the number of animals in a group.

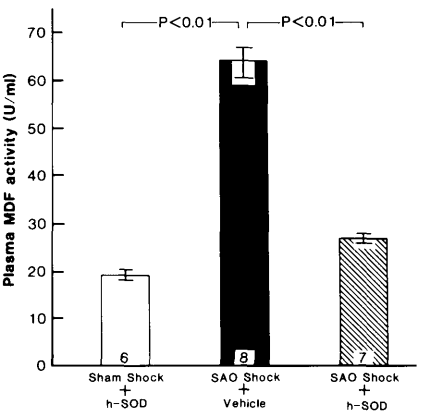

FIG. 4. Plasma myocardial depressant factor (MDF) activities at the end of the experiment in the three experimental groups of sham-shock and SAO shock rats. Bar heights are means; brackets indicate ±SEM. The numbers in the bars indicate the number of animals in a group.

treated SAO shock rats exhibited plasma MDF activities which were significantly lower than those of untreated SAO shock rats ($P < 0.01$) and not significantly different from sham shock rats.

Intravascular fluid volume loss is a contributing factor in the pathophysiology of SAO shock. In order to assess net fluid volume loss from the intravascular compartment, we determined the initial and final hematocrits. The hematocrit at the beginning of the experiment was comparable in the three experimental groups and ranged from 44 ± 1.2 to 45 ± 0.6%. SAO shock rats receiving only the vehicle exhibited a large increase in the hematocrit reaching a value of 65 ± 1.6% at the end of the experiment. In contrast, SAO shock rats treated with h-SOD exhibited a significantly lower final hematocrit of 58 ± 2.4% ($P < 0.05$ from vehicle).

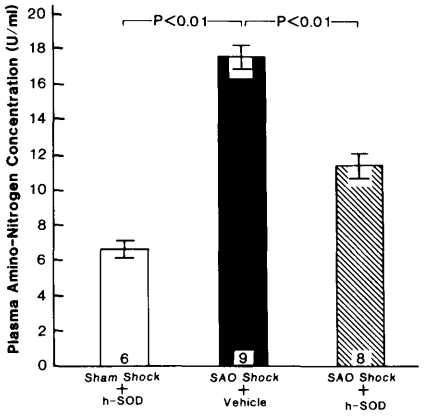

FIG. 3. Plasma amino-nitrogen concentrations at the end of the experiment in the three experimental groups of sham shock and SAO shock rats. Bar heights are means; brackets indicate ±SEM. The numbers in the bars indicate the number of animals in a group.

All sham shock rats survived the full 160-min experimental protocol. In the SAO shock group receiving the vehicle, only one out of nine rats (11%) survived at the end of the observation period. In contrast, treatment with h-SOD significantly improved the survival rate of SAO shock rats, with seven out of eight rats (88%) surviving the full experimental protocol ($P < 0.01$ from vehicle).

Discussion. Occlusion of the major blood vessels supplying the splanchnic region followed by reperfusion uniformly produces a severe form of circulatory shock having a high mortality rate (1, 2). Some of the mechanisms involved in the pathophysiology of SAO shock have already been well established. Thus, a relatively short period of splanchnic ischemia (i.e., less than 30 min in the rat) (16) results in parenchymal and microvascular damage manifested by intestinal mucosal ulcerations, increased microvascular permeability, tissue edema, and fluid loss outside of the vascular compartment (17,

18). The severity of these processes is relative to the duration of the ischemia and is aggravated after reperfusion in areas where irreversible damage has not developed (19). Splanchnic ischemia and the resulting hypoxia and acidosis of the pancreas and the small intestine cause massive release of lysosomal hydrolases, enhanced proteolysis, and production of the cardiodepressant substance MDF (1–3). Additionally, depression of myocardial function is also important in the development of SAO shock, and is believed to be caused by the combined effects of cardiotoxic substances, intravascular fluid volume loss, and possibly other mechanisms (e.g., coronary and peripheral vasoconstriction, increased pulmonary vascular resistance) which may involve other vascular mediators (2, 20–23).

The involvement of oxygen-derived free radicals in mediating the pathophysiological processes occurring in splanchnic ischemia has already been established. The mucosal villi of the intestine contain the highest concentration in the body of xanthine oxidase, a key enzyme for oxygen radical production (4). Furthermore, intra-arterial infusion of a free radical-generating enzymatic system increased intestinal microvascular permeability to a degree comparable to that induced by ischemia (24, 25). These changes can be ameliorated by pretreatment with radical scavengers (24, 25) or with the xanthine oxidase inhibitor, allopurinol (8). Also, pretreatment with free radical scavengers (e.g., SOD, DMSO) has been shown to attenuate the morphologic changes associated with splanchnic ischemia (4, 26). Thus, it appears that oxygen-derived free radicals are important mediators of the reperfusion injury following partial ischemia of the splanchnic region or total occlusion of splanchnic blood flow without total tissue damage. It has been proposed that under these pathological conditions, increased production of oxygen-free radicals results in peroxidation of lipid components of cellular and subcellular membranes and degradation of constituents of the basement membrane and extracellular matrix (i.e., hyaluronic acid and collagen). (8). These membrane lytic processes may contribute to the characteristic ischemia–reperfusion-induced increased vascular permeability and mucosal damage, as well as to the increased lysosomal disruption observed in SAO and other types of circulatory shock. Oxygen-derived free radicals could arise from the action of xanthine oxidase. In this regard, the rat liver is a very rich source of this enzyme (27).

In the present study, we studied the effects of human superoxide dismutase (h-SOD) produced by molecular biological technology (11). h-SOD given post-treatment at the end of the ischemic period, concomitantly with reperfusion, exerted beneficial effects in rats during a very severe model of SAO shock. MABP was maintained at significantly higher values during the postreperfusion period in h-SOD-treated shocked rats compared to SAO rats receiving the vehicle. Furthermore, our data show that SAO shock rats treated with h-SOD also exhibit lower plasma amino-nitrogen concentrations as well as lower plasma activities of cathepsin D and MDF. The source of the plasma cathepsin D has been shown to be largely from the ischemic pancreas and liver (1) although circulating granulocytes may also contribute to the elevated acid hydrolase activity. The improved circulatory and metabolic status of SAO rats treated with h-SOD was followed by a significantly improved survival rate when compared with SAO shock rats receiving the vehicle (i.e., 11% vs 88%).

The mechanism of the protective effect of h-SOD in SAO shock probably involves several components. Since h-SOD was given after the ischemic period, it could only ameliorate pathophysiologic processes occurring after reperfusion. It seems likely that protection against reperfusion-induced microvascular permeability changes and mucosal damage could ameliorate intravascular volume loss and improve overall tissue perfusion. The significantly improved hematocrit in h-SOD-treated SAO rats is consistent with this suggestion. Furthermore, the marked increase in hematocrit values exhibited by untreated SAO shock rats indicates a significant increase in blood viscosity which is clearly detrimental to organ perfusion and myocardial function. Additionally, improved mucosal and microvascular integrity could indirectly lead to amelioration of tissue and plasma proteolysis and toxic factor produc-

tion during SAO shock. A direct antiproteolytic effect of h-SOD has not been ruled out in our study, and requires further investigation. The attenuation of plasma proteolysis and MDF production is known to be related to improved survival in circulatory shock (28). The cathepsin D data suggest that h-SOD exerts a statistically significant lysosomal membrane stabilizing effect. This effect is probably secondary to better tissue perfusion and hemodynamic status of h-SOD-treated rats.

In summary, we have shown a protective effect of human superoxide dismutase in SAO shock. Post-treatment with h-SOD maintained postreperfusion MABP, decreased the plasma accumulation of free amino-nitrogen compounds, cathepsin D activity, and the cardiodepressant factor MDF, and resulted in increased survival rate. These effects may be mediated by amelioration of microvascular and parenchymal injury induced by oxygen-derived free radicals after reperfusion of the ischemic splanchnic region. These results are consistent with the hypothesis that oxygen-derived free radicals play an important role in the pathophysiology of SAO shock.

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