Recombinant Human-type SOD Attenuates Circulatory Disorders after Reperfusion of Splanchnic Organs in Rats

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Oxygen free radicals (OFRs) have been reported to play pivotal roles in the pathogenesis of cell damage induced by ischemia and reperfusion. The efficacy of recombinant human superoxide dismutase (rh-SOD) in the treatment of circulatory disorders after reperfusion of the splanchnic area was investigated in rats. All rats died within 3 hours after release of 60-min superior mesenteric artery occlusion (SMAO) when no treatment was given. Animals which received rh-SOD, 2 mg·100g^{-1}BW, at reperfusion followed by a continuous infusion of rh-SOD 0.67 mg·100g^{-1}BW·hr^{-1}, exhibited prolonged survival times compared with no treatment rats (231 ± 35 min and 149 ± 43 min, respectively). Mean blood pressure in rats treated with rh-SOD was higher than in controls after reperfusion, and was concomitant with improvement in splanchnic perfusion. The results suggest excessive activity of OFRs in reperfused organs and a possible scavenging effect of rh-SOD as a means of eliminating them. (Key words: shock, oxygen free radicals, superoxide dismutase, survival rate, hemodynamics)

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It is well known that ischemia followed by reperfusion severely damages organs[1–3]. The pathology involves ischemic diseases, shock, organ transplantation and so on[4–6]. The direct mechanism by which cell injuries are produced is believed to be accumulation of oxygen free radicals (OFRs)[7–9]. Organ damage might be prevented if methods of eliminating OFRs could be introduced into clinical field. The most promising method is an exogenous supply of superoxide dismutase (SOD), an enzymatic OFR scavenger[10,11]. Recently, an industrial cell line has been established to produce recombinant human-type SOD (rh-SOD) using techniques based on gene recombination, permitting a commercial supply of rh-SOD in the form of an extremely purified preparation. The purpose of this study was to investigate its effects on the hemodynamics of rats with superior mesenteric artery occlusion shock (SMAO shock).

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Materials and Methods

Male Wister-strain rats weighing about 250 g were used. The animals were divided into three groups. Group 1, subjected to anesthesia and laparotomy alone, served as the sham-operated control (n = 7). SMAO shock

was produced after anesthesia and laparotomy in Group 2, but no treatment was administered (n = 11). Group 3 underwent the same procedure as Group 2 but was infused with rh-SOD to treat the shock (n = 11).

a. Preparation of the shock model

The rats were anesthetized with chloral hydrate 36 mg·100g^{-1}BW i.p. A 5Fr teflon tube was inserted into the trachea in order to maintain an airway. Respiration was controlled using a small animal ventilator (Type-SAV, Natsume Manufacturing Co. Ltd., Tokyo), after immobilization with pancuronium bromide 200 μg·100g^{-1}BW i.v. Respiration was adjusted to a rate of 50 cpm and a tidal volume of 1 ml·100g^{-1}BW, so that Pa_{CO_2} was approximately 35 mmHg. A 24G indwelling catheter was introduced into a tail vein to allow administration of saline and drugs. In addition, a small incision was made in the neck to enable insertion of a 24G catheter into the carotid artery. This catheter was used to record blood pressure and to sample blood. Animals from all groups were subjected to laparotomy along the median line. After careful hemostasis heparin 200 units·100g^{-1}BW was injected intravenously as the anticoagulant. The superior mesenteric artery (SMA) were occluded at their origin using bulldog-type clips when respiratory and circulatory conditions were stabilized. When cessation of pulsation on the intestinal wall and a change in bowel color were verified, the abdomen was closed with continuous sutures. After 60 min of occlusion the clips were removed and reperfusion was confirmed by the reappearance of pulsations and recovery of organ color. The incision was securely closed again after confirming the absence of hemorrhage.

b. Experimental protocol

The animals in Group 1 were anesthetized and the abdomen was opened without SMA occlusion as a sham-operated control. Saline was infused at a rate of 1 ml·h^{-1} for 5 hrs.

The rats in Group 2 were subjected to shock by SMAO for 60 min and reperfusion. Saline was infused at a rate of 1 ml·h^{-1} after occlusion until the end of the experiments. The rats in Group 3 were subjected to the SMAO shock similar to the rats in Group 2. The rats received rh-SOD 2 mg·100g^{-1}BW dissolved in 1 ml of saline as i.v. bolus upon release of the occlusion and were infused with rh-SOD dissolved in 1 ml of saline at a rate of 0.67 mg·100g^{-1}BW·h^{-1} until completion of the experiment. The rh-SOD offered by Nippon Kayaku Co., Ltd, Tokyo, Japan, was produced in genetically transformed bacteria. One mg of rh-SOD contained 3,000 units of specific activity.

Mean arterial blood pressure (MABP) was determined and recorded on a polygraph throughout the experiments. The survival times in Group 2 and 3 were also recorded. Blood was collected before SMAO, and 1, 2 and 3 hr after release of the occlusion in Groups 2 and 3. Blood was collected in Group 1 at times corresponding to those in the Groups 2 and 3.

c. Measurements

The catheter in the carotid artery was connected to a pressure transducer and MABP was recorded on a polygraph, Hewlett-Packard Model 78342-A. The blood withdrawn was used to determine blood gases (pH, Pa_{O_2}, Pa_{CO_2}, BE), hemoglobin (Hb), electrolyte concentrations (Na, K), lactate (L) and pyruvate (P). Blood gases and Hb were analyzed using a blood gas analyzer, Radiometer Co., Ltd. Model ABL-3, and electrolyte concentrations using a Nova-Biomedical Co., Ltd. Model NOVA-2. L and P were measured twice before occlusion and 1

Table 1. Blood gas values in 3 groups during experiments

		control	1 hr	2 hrs	3 hrs
pH	Group 1	7.44 ± 0.06	7.34 ± 0.09	7.27 ± 0.15!	7.29 ± 0.11
	Group 2	7.40 ± 0.05	7.05 ± 0.11!!☆☆	7.00 ± 0.07!!☆☆	6.98 ± 0.10!!☆☆
	Group 3	7.40 ± 0.04	7.18 ± 0.07!!☆☆	7.11 ± 0.10*!!☆☆	7.02 ± 0.12!!☆☆
Pa_{O_2} (mmHg)	Group 1	95.3 ± 15.0	96.3 ± 10.5	104.2 ± 14.2	106.6 ± 7.8
	Group 2	94.3 ± 14.8	99.0 ± 21.0	109.5 ± 17.0	107.8 ± 6.9
	Group 3	93.6 ± 12.0	102.8 ± 13.6	108.3 ± 19.4	103.1 ± 22.8
Pa_{CO_2} (mmHg)	Group 1	36.3 ± 4.0	38.0 ± 2.2	38.8 ± 4.1	40.2 ± 3.6
	Group 2	33.9 ± 3.2	41.5 ± 7.3!	42.7 ± 11.2	40.8 ± 10.0
	Group 3	34.1 ± 4.3	38.6 ± 8.1!	40.4 ± 8.9	40.3 ± 9.4
BE (mEq/ℓ)	Group 1	−5.0 ± 2.2	−5.6 ± 1.8	−6.5 ± 2.0!	−6.8 ± 2.3!
	Group 2	−4.7 ± 2.5	−17.5 ± 3.4!!☆☆	−20.5 ± 2.9!!☆☆	−21.6 ± 3.5!!☆☆
	Group 3	−5.3 ± 1.7	−12.6 ± 3.0*!!☆☆	−16.7 ± 4.8*!!☆☆	−20.9 ± 6.2!!☆☆

"!" and "!!" show significant differences as compared with control value at P value less than 0.05 and 0.01, respectively. "*" shows a significant difference between Groups 2 and 3 at P value less than 0.05. "☆☆" shows a significant difference as compared with Group 1 at P value less than 0.01.

Table 2. Plasma Na and K in 3 groups

		control	1 hr	2 hrs	3 hrs
Na (mEq/ℓ)	Group 1	132.1 ± 6.8	129.6 ± 2.0	131.0 ± 2.3	132.3 ± 2.7
	Group 2	128.6 ± 2.6	125.2 ± 3.7	126.6 ± 3.9	128.7 ± 4.9
	Group 3	129.3 ± 1.7	126.2 ± 1.3	128.0 ± 3.0	132.8 ± 2.0
K (mEq/ℓ)	Group 1	4.9 ± 0.6	4.6 ± 0.5	4.6 ± 0.9	4.4 ± 1.1
	Group 2	5.0 ± 0.1	6.5 ± 1.7!☆	6.8 ± 1.3!☆	7.0 ± 1.5!☆
	Group 3	5.2 ± 0.9	5.6 ± 0.9*!☆	5.7 ± 0.8*!☆	6.0 ± 1.1*!☆

"!" shows a significant difference as compared with control value at P value less than 0.05. "☆" shows a significant difference as compared with Group 1 at P value less than 0.05. "*" shows a significant difference between Groups 2 and 3 at P value less than 0.05.

hr after release, because it took a great deal of blood to perform the analyses by the lactate oxidase and pyruvate oxidase methods. The status of anerobic metabolism was expressed in the form of the L/P ratio.

d. Statistics

The numerical results obtained were represented as mathematical means ($\bar{X}$s) ± standard deviations (SDs). Differences among more than 3 groups were tested by one-way analysis of variance. Differences between 2 groups were analyzed by t-tests. The discrete data obtained were expressed by percent and the differences between 2 groups were assessed by chi-square test. P values less than 0.05 were considered statistically significant.

Results

There were no differences among the 3 groups with respect to body weight, MABP, blood gases, Hb, plasma electrolytes, L, P or L/P ratio before the experiments as shown in the tables 1 and 2, and figure 1, 3 and 4.

Changes in MABP are shown in figure 1. No changes were observed in

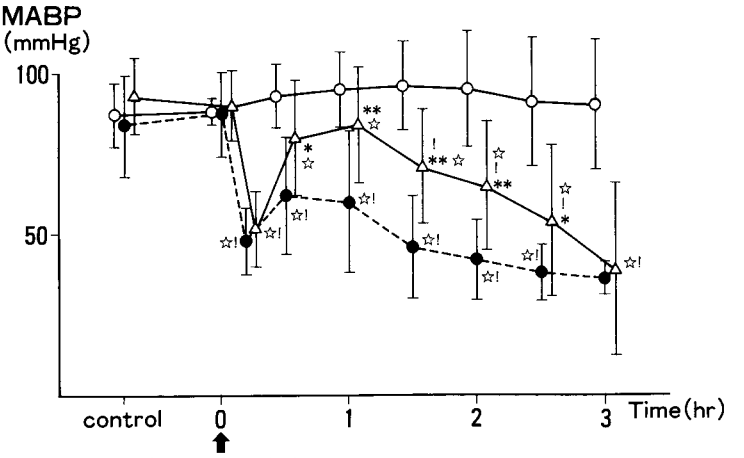

Fig. 1. Changes in mean arterial pressures in 3 groups during experiments.

○–○; Group 1, ●–●; Group 2 and △–△; Group 3. "!" shows a significant difference as compared with control value at P value less than 0.05. "☆" shows a significant difference as compared with Group 1 at P value less than 0.05. "*" and "**" show significant differences between Groups 2 and 3 at P value less than 0.05 and 0.01, respectively.

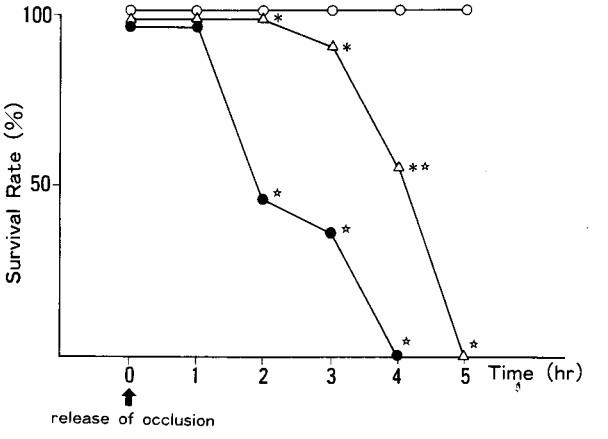

Fig. 2. Survival rates of rats during experiments.

○–○; Group 1, ●–●; Group 2 and △–△; Group 3. "☆" shows a significant differencne as compared with Group 1 at P value less than 0.05. "*" shows a significant difference between Groups 2 and 3 at P value less than 0.05.

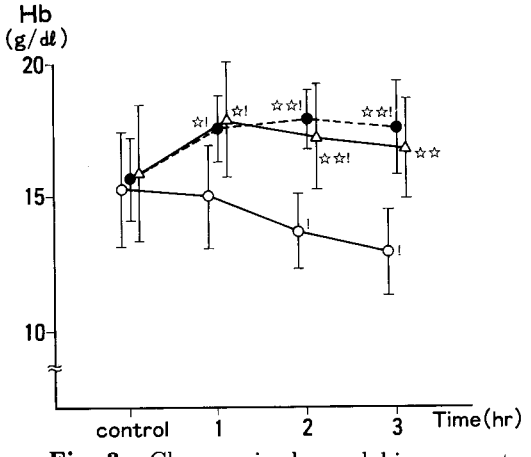

Fig. 3. Changes in hemoglobin concentration in 3 groups during experiments.

○–○; Group 1, ●–●; Group 2 and △–△; Group 3. "!" shows a significant difference as compared with control value at P value less than 0.05. "☆" and "☆☆" show significant differences as compared with Group 1 at P value less than 0.05 and 0.01, respectively.

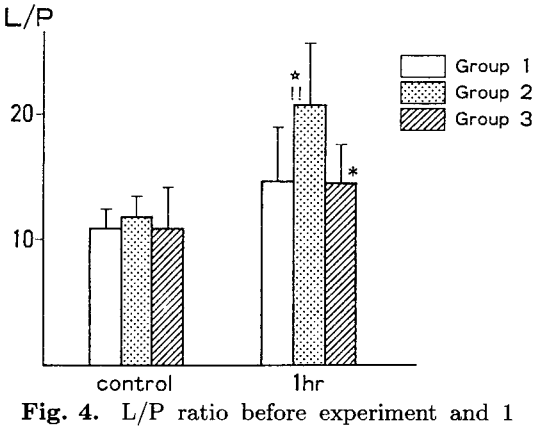

Fig. 4. L/P ratio before experiment and 1 hr after reperfusion. "!!" shows a significant difference as compared with control value at P value less than 0.01. "☆" shows a significant difference as compared with Group 1 at P value less than 0.05. "*" shows a significant difference between Groups 2 and 3 at P value less than 0.05.

Group 1 at any time during the experiment. The animals in Group 2 and 3 showed an abrupt decline to around 50 mmHg immediately after release of the occlusion. MABP in both groups recovered within 30 min. The recovery in blood pressure was marked in Group 3 and significantly different from Group 2 throughout the experiments.

Figure 2 shows the survival rates for the 3 groups. Animals in Group 1 survived over 5 hrs. The survival rates in Group 2 were 46% (5/11), 36% (4/11) and 0% (0/11), 2, 3 and 4 hrs respectively after release of the occlusion. The survival rates in Group 3 were 100% (11/11), 91% (10/11) and 55% (6/11), 2, 3 and 4 hrs respectively after release of the occlusion. Significant differences were observed between the 2 groups, 2, 3 and 4 hrs after reperfusion. Mean survival times were 149 ± 43 min in Group 2 and 231 ± 35 min in Group 3. A significant difference was found between the two.

Table 1 shows the results of arterial blood gas analysis. Slight but significant decreases in pH and buffer base were observed in Group 1 after 3 hrs of setting-up. The pH and buffer base in Groups 2 and 3 declined abruptly after the release of occlusion and were significantly different from Group 1 after 1 hr of reperfusion. Significant differences were also observed in pH and buffer base between Group 2 and 3 after reperfusion.

Hb decreased significantly in Group 1 at a time equivalent to 2 hrs after reperfusion in Groups 2 and 3. The Hbs in Groups 2 and 3 on the other hand increased markedly after 1 hr of reperfusion, and were significantly different from the first group. No difference was observed between Groups 2 and 3, however (fig. 3).

Electrolyte concentrations underwent some changes (table 2). Plasma Na underwent no changes in any of the 3 groups at any time during the experiments. Although plasma K did not change in Group 1, it did increase significantly after 1 hr of reperfusion in Groups 2 and 3. There were significant differences in plasma K level between Groups 2 and 3, reflecting attenuation of the elevation in K level in Group 3.

The L/P ratio rose significantly in Group 2 after reperfusion, and there was a significant difference between Groups 2 and 3 (fig. 4).

Discussion

It has been observed that reperfusion of splanchnic organs after temporary ischemia induces a severe shock state in experimental animals. The mechanism by which shock is induced is believed to be depletion of plasma components due to an excessive increase in the permeability of capillaries of ischemic regions[10,11]. Granger et al.[12] reported an evidence of enhanced capillary permeability in rat mesentery after 60 min of splanchnic ischemia. Dawidson et al.[13] confirmed depletion of plasma water when they could prevent circulatory collapse by massive infusion of crystalloid solution.

The precise mechanisms of enhanced vascular permeability remain to be discovered. Production of vasoactive substances, absorption of endotoxin from the intestinal canal, release of lysosomal enzymes from intracellular vesicles and/or release of specific substances such as MDF and Clows' factor have been suggested as causative factors[14].

Recently the cellular damage produced by ischemia and reperfusion has been proposed as a main factor in enhanced capillary permeability by Korthius et al[15]. They observed the enhanced capillary permeability in canine skeletal muscle associated with accumulation of OFRs. Parks et al.[16] and McCord[17] presented the hypothesis that xanthine oxidase transformed from xanthine dehydrogenase by pro-

tein kinase produced OFRs by reducing ground state of oxygen to the superoxide anion. OFRs damage capillary endothelial cells and induce enhanced permeability of capillary wall[18].

Localized injuries such as trauma and ischemia result in the release of large quantities of prostanoids and eicosanoids into circulating blood[19]. The rise in LTB4 appears to be correlated with a reduction in circulating leukocytes and to subsequent leukocyte respiratory burst activation[20]. This leukosequestration was prominent in splanchnic organs damaged by ischemia, inducing protease degranulation and superoxide anion release[21]. Leukocyte proteases including elastase and OFRs attack endothelial cells and destroy them as reported by Grisham et al[22].

If OFRs play a pivotal role in the enhanced capillary permeability of splanchnic organs, methods of inhibiting their activity might prevent hemodynamic disorders in animals with temporary splanchnic ischemia. Measures proposed have consisted of inhibition of xanthine oxidase activity and exogenous supply of enzymatic scavengers and/or chemical quenchers. Kunimoto et al.[23] and Ogawa et al.[24] reported the effectiveness of SOD in improving the survival rates of rats with lethal endotoxemia and temporary splanchnic ischemia.

Recently rh-SOD has been successfully produced as a result of the technique of genetic recombination. There is no possibility of an anaphylactic reaction by rh-SOD when used clinically. However, Michelson et al.[25] earlier suggested the lack of scavenging activity in rh-SOD and this problem remained unsolved. The present study was undertaken to determine the effects of rh-SOD in an animal model of ischemia and reperfusion.

The results of the present study showed an improvement in circulation and a prolongation of survival time in rats infused with large amounts of rh-SOD. The finding that potassium concentration and L/P ratio in the blood of rats with rh-SOD were maintained at levels lower than the control suggests the attenuation of organ injuries in the splanchnic area. SOD dismutates O_2^- to H_2O_2 very quickly, and the glutathione peroxidase present in large amounts in the cell converts H_2O_2 to H_2O. Rh-SOD has a mean molecular weight of 32,000 daltons and is excreted into urine by the kidney. It requires a large amount of rh-SOD to maintain effective concentrations in the blood. Ph-SOD in a dose of 2 $mg \cdot 100g^{-1}BW$ was injected upon release of SMA occlusion and 0.67 $mg \cdot 100g^{-1}BW \cdot hr^{-1}$ was administered continuously in the present study.

The main site of OFR production is the cytoplasm in ischemic and reperfused organs. Rh-SOD is not believed to penetrate into the cell. Ability to scavenge OFRs in the blood, however, plays an important role in the elimination of intracellular OFRs. Intravenous administration of exogenous rh-SOD is considered effective in attenuating the activity of OFRs in reperfused organs.

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