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Effect of Polyethylene Glycol Conjugated Superoxide Dismutase on Hepatic Ischemia/Reperfusion Injury in Rats

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Superroxide anion radical (O_2^-) has been suggested as a causative factor of ischemia/reperfusion injury to the liver. Superxide dismutase (SOD)is a specific scavenger for O_2^-, but its elimination half life in the blood is about five min. Polyethylene glycol conjugated SOD (PEG-SOD) has a chracteristics of long half life (14hr) in the circulating blood and low immunogenicity. In the present study, we compared the effect of PEG-SOD to conventional SOD in protecting the ischemia/reperfusion injury to the liver. In rats with an occluded inflow against 70% of the liver for 30min followed by 30min reperfusion, elevations of serum aspartate aminotransferase and alanine aminotransferase,and lipid peroxide concentrations in the liver were not significantly inhibited by intravenous administration of PEG-SOD, compared to those treated with conventional SOD. These results indicate that sustained presence of radical scavenger activity in the circulating blood has no more beneficial effects on hepatic ischemia/reperfusion injury than its temporary presence when reperfusion begins.

Introduction

Hepatic ischemia/reperfusion injury is a serious problem, e.g., hepatic failure after shock, liver transplantation, or liver surgery. Superoxide anion radical (O_2^-) has been implicated in the pathogenesis of tissue injury related to ischemia/reperfusion[1]. Superoxide dismutase (SOD), a specific scavenger for O_2^-, has been evaluated using a variety of experimental models. However, the preventive effect of SOD against ischemia/reperfusion injury remains controversial. One of the disadvantage of SOD is a short elimination half life in the circulating blood (about five min), because SOD has a low molecular weight (32, 000) and is easily filtrated in the kidney and excreted into the urine[2]. Polyethylene glycol conjugated SOD (PEG-SOD) has a long half life in the circulating blood (about 14 hr) and low immunogenicity[3]. In the present study, the effect of PED-SOD on hepatic ischemia/reperfusion injury was investigated to compare events related to the use of conventional SOD.

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Materials and Methods

Chemicals

SOD was provided by Asahi Chemical Industry Co., Shizuoka, Japan. PEG-SOD was provided by Takeda Chemical Industry Co., Osaka, Japan. Physicochemical properties of SOD and PEG-SOD are shown in Table 1. The molecular weight of SOD, estimated by high performance liquid chromatography, was 32, 000 and that of PEG-SOD was 100, 000. The remaining enzymatic activity, assayed by cytochrome C reduction method[4] was 52% of conventional SOD.^{111}Indium chloride ($[^{111}In]InCl_3$: Nihon Medi-Physics Co., Takarazuka, Japan) labeling of SOD and PEG-SOD was performed according to the method of Hnatowich et al.[5].

Animals

Male Wistar rats (200-300g), purchased from Charles River Japan (Atsugi, Japan), were bred in the Laboratory Animal Center for Biochemical Research, Nagasaki University School of Medicine. These animals were fasted but had free access to water 12 hours before the experiments.

Table 1. Physicochemical properties of SOD and PEG-SOD

	Molecular Weight[a]	Modified Amino Groups(%)[b]	Remained Enzymatic Activity(%)[c]
SOD	32000	0	100
PEG-SOD	100000	24.0	52.0

a):estimated by high performance liquid chromatography
b):assayed by the cytochrome C reduction method
c):determined by the trinitrobenzene sulfonic acid method

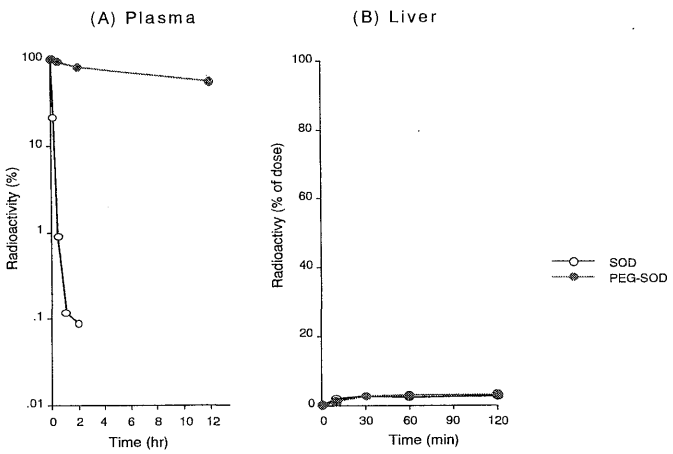

Fig 1. (A) Plasma concentration of [^{111}In] SOD and PEG-SOD following intravenous injection (1mg/kg as SOD, n = 3)
(B) Liver accumulation of [^{111}In] SOD and PEG-SOD following intravenous injection (1mg/kg as SOD, n = 3)

Pharmacokinetic Assays

^{111}In radiolabeled SOD or PEG-SOD was injected into the tail vein of 3 rats. At an appropriate time period after administration, blood was collected from the abdominal aorta and then, the liver was excised, rinsed with saline, weighed, and subjected to assay. ^{111}In radioactivities in the plasma and liver samples were countered with a well NaI-scintillation counter (ARC-300, Aloka, Tokyo).

Hepatic Ischemia/Reperfusion

The rats were anesthetized with sodium pentobarbital intraperitoneally (50 mg/kg). After heparinization (50 IU/kg), laparotomy was performed with transverse incision and the hepatic hilum was exposed. According to the method of Hasselgren et al.[6], the blood vessels to the left and median lobes of the liver were occluded for 30 min with a vascular clamp. Ten sec prior to release of this clamp, each treatment was given via tail vein, as follows.

Group I : 0.5 ml of normal saline (n = 6).
Group II : 10, 000 units/kg of SOD dissolved in 0.5 ml of normal saline (n = 6).
Group III : 10, 000 units/kg of PEG-SOD dissolved in 0.5 ml of normal saline (n = 6).

Thirty min after the restoration of hepatic blood flow, blood samples were obtained from the abdominal aorta to determine serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Then, the liver was perfused with 0.05 M phosphate buffer solution (pH 7.4) and the postischemic portion of the liver was frreeze-clamped and stored in liquid nitrogen. Quantitative estimation of lipid peroxide (LPO) in the liver was made according to the procedure of Masugi and Nakamura[7]. SOD activity was assayed by the cytochrome C reduction method[4]. Light microscopic examination of the postischemic portion of the liver in all groups was carried out.

Statistical Analyses

All results are expressed as mean ± SD. Statistical differences between means were evaluated with Student's t test (2 group comparison) or one way analysis of variance followed by Fisher's PLSD test (3 group comparison). $P < 0.05$ was considered significant.

Results

Pharmacokinetics of SOD and PEG-SOD

Fig.1 shows radioactivities of ^{111}In in the plasma and liver following intravenous administration of ^{111}In radiolabeled SOD or PEG-SOD at a dose of 1mg as SOD/kg. Conventional SOD was rapidly cleared from blood circulation and its half life was only five min. The plasma half life of PEG-SOD was dramatically increased to 14hr. However, PEG-SOD was also negligibly incorporated into the liver as well as conventional SOD.

Effects of SOD and PEG-SOD on Ischemia/Reperfusion Injury

Table 2 shows a summary of the laboratory results of all the groups. Among three groups, there was no significant difference in serum AST and ALT, and LPO concentration in the liver. However, these parameters tended to be attenuated by administration of SOD and PEG-SOD, almost equally. Light microscopic findings showed dilatation of sinusoid and degeneration with vacuolization of hepatic cells in the area of central vein in Group I (Fig.2-A). In Group II and III, these findings were mild (Fig.2-B, C).

Table 2. Effects of SOD and PEG-SOD on hepatic ischemia/reperfusion injury

	Group I (normal saline,n = 6)	Group II (SOD,n = 6)	Group III (PEG-SOD,n = 6)
serum AST (IU/l)	475±63	343±133	340±177
serum ALT (IU/l)	160±53	129±49	116±58
tissue SOD (U/mgProt)	26.7±2.4	24.3±2.5	25.1±3.1
tissue LPO (nmolMDA/mgProt)	0.40±0.08	0.36±0.08	0.36±0.06

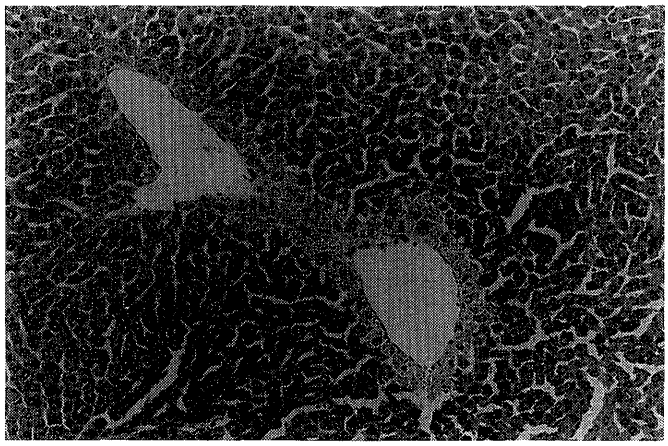

Fig. 2 (A)

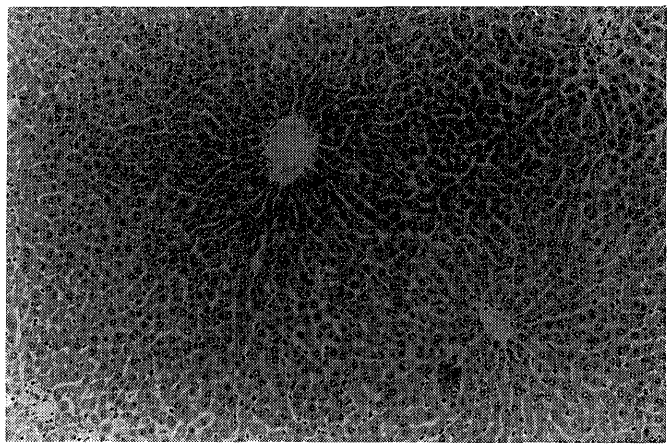

Fig. 2 (B)

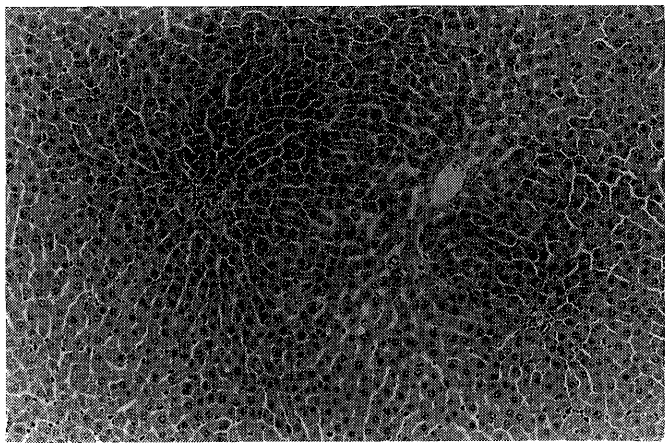

Fig. 2 (C)

Fig 2. Light microscopic findings after ischemia/reperfusion. Sinusoidal dilatation and hepatic cell degenaration in the area of centrao vein in Group I (A); Mild these findings in Group II (B) and Group III (C)

Discussion

Recent studies have suggested that oxygen free radicals play a significant role in ischemia/reperfusion injury [1]. In the liver ischemia/reperfusion model, LPO formation may have been induced from chain reaction of oxygen free radicals caused by a large amount of oxygen inflow during reperfusion. Cellular damage of the liver caused by ischemia/reperfusion is assumed to be at least in part due to lipid peroxidation in biomembrane [8]. SOD is a specific scavenger for O_2^-, however, it is not sufficient to prevent this injury because of its short half life in the circulating blood and low affinity to tissue [2].

In the present study, native SOD and PEG-SOD inhibited the elevation of AST and ALT in serum and LPO concertration in liver tissue. The inhibitory efficacy of AST and ALT on this injury was almost the same as that of SOD. On the other hand, SOD activity in liver tissue did not differ among three groups. It means that sustained presence of SOD activity in the circulating blood has no beneficial effects. The possible reasons are [I] oxygen free radicals are produced only at the time of beginning of reperfusion, [II] most of radicals are not produced in the circulating blood.

Bolli et al. [9] reported that release of oxygen free radicals continued three hr after reperfusion. However,it is argued that oxygen free radicals produced only in early phase after reperfusion participate in ischemia/reperfusion injury [10].

It is uncertain as to which type of cell, endothelial, Kupffer or parenchymal cells in the liver or neutrophils in the vasculature, is the main source of superoxide anion radicals. It is recognized that not only O_2^- produced by neutrophil itself [11], but also the neutrophil-endothelial cell adhesion interaction [12] is a initial step in the pathogenesis of ischemia/reperfusion injury. Our results suggest that oxygen free radicals released from neutrophils into the circulating blood do not play a significant role in hepatic ischemia/reperfusion injury.

PEG-SOD has a potential therapeutic efficacy on injuty related to superoxide anion radicals because of its long half life in the circulating blood and low immunogenicity. In fact, PEG-SOD has been evaluated using in variety of experimental models [13-15]. However, in the present study, PEG-SOD had no more efficacy than conventional SOD.

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