

Effect of Lecithinized-Superoxide Dismutase on the Rat Colitis Model Induced by Dextran Sulfate Sodium

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ABSTRACT—Lecithinized-superoxide dismutase (PC-SOD), which is synthesized with a lecithin derivative bound covalently to recombinant human Cu,Zn-SOD, has a longer half-life in blood and higher cell affinity than unmodified SOD. The effects of PC-SOD were evaluated using the rat ulcerative colitis model induced by 3% dextran sulfate sodium. Intravenous injection of rats with 0.5 or 1 mg/kg of PC-SOD suppressed the progression of bloody stools, the formation of erosion, and the infiltration of the colon with inflammatory cells. Furthermore, it also reduced the increase of leukocytes in blood. Thus, PC-SOD may have therapeutic potential in the treatment of ulcerative colitis.

Keywords: Lecithinized-superoxide dismutase, Dextran sulfate sodium, Ulcerative colitis

It has been reported that oxygen free radicals are related to a deterioration in inflammatory bowel disease (IBD) (1–3). Therefore, radical scavengers such as superoxide dismutase (SOD) were expected to be therapeutic drugs for IBD; however, to date, none have been put to practical use. The weak point of SOD is that it has a half-life of only several minutes in plasma and has a low tissue affinity. One way to overcome such disadvantages is to incorporate SOD into a drug delivery system. Mizushima et al. (4) have developed a drug delivery system of lipid microspheres that consist of soybean oil surrounded by lecithin. Some investigators, including Mizushima and Igarashi (5), have found that lecithin is highly cytotropic and safe. Igarashi et al. developed lecithinized-SOD (PC-SOD), which is synthesized with a lecithin (phosphatidylcholine, PC) derivative bound covalently to recombinant human Cu,ZnSOD (6). PC-SOD has a longer half-life in plasma, a higher cell affinity and has more pharmacological potency than unmodified SOD (6–8). Additionally, the lecithin derivative has no pharmacological potency (6).

In the present study, we investigated the therapeutic effect of PC-SOD or salazosulfapyridine (SASP), which is the therapeutic drug for IBD, on the rat colitis model induced by ingestion of dextran sulfate sodium (DSS) (9).

Healthy seven-week-old male Wistar rats (SPF) (Charles River Japan Inc., Yokohama) were used in the experiments. These rats were housed in wire-mesh bottom cages

in a room at a controlled temperature, humidity and light, with food and water available ad libitum. During breeding, plain tap water was supplied. During examinations, the normal group received purified water supplied in bottles and the other groups received 3% DSS solution supplied in bottles. PC-SOD (Lot No. FU95002; Asahi Glass Co., Ltd., Tokyo) in which four molecules of lecithin derivative are covalently bound to each dimer of recombinant human SOD was used in the experiments. It was diluted in 5% mannitol (Wako Pure Chemical Industries, Ltd., Osaka) and prepared for doses of 0.25, 0.5, 1 or 2 mg/2 ml/kg. SASP (Sigma Co., St. Louis, MO, USA) was suspended in 0.5% carboxy methyl cellulose sodium solution (Maruishi Pharmaceutical Company, Tokyo) and prepared for a dose of 25 mg/5 ml/kg. DSS (mean MW: 5000; Wako Pure Chemical Industries, Ltd.) was dissolved in purified water and a 3% solution was prepared. A total of 114 rats were randomly placed into seven groups, and they were housed individually. The normal group (n=6) was given purified water, and the other groups were given 3% DSS for 10 days. PC-SOD (0.25–2 mg/2 ml/kg, 18 rats/group) was injected intravenously daily from day 0 to day 9. SASP (25 mg/5 ml/kg, n=18) was orally administered daily during the same period. The stools were observed everyday. The quantity of 3% DSS ingested was determined, and 3% DSS was supplied every second day. The symptom of bloody stool was graded using a bloody stool score. This

Table 1. Time course of bloody stool score in the rat colitis model treated with PC-SOD

Drug	The day for DSS ingestion									
	1	2	3	4	5	6	7	8	9	10
Control	0±0	0.06±0.06	0.17±0.06	0.22±0.06	0.44±0.06	0.78±0.06	1.50±0.06	1.83±0.06	1.89±0.06	2.00±0.06
PC-SOD (mg/kg)	0.25	0±0	0.06±0.06	0.11±0.08	0.17±0.09	0.39±0.12	1.06±0.19	1.28±0.14 ^a	1.65±0.15	1.82±0.10
	0.5	0±0	0±0	0±0	0.06±0.06 ^a	0.33±0.11	0.72±0.11 ^b	1.00±0.16 ^b	1.44±0.15	1.83±0.09
	1	0±0	0±0	0±0	0.11±0.08	0.17±0.09	0.39±0.14	0.94±0.13 ^a	1.39±0.16	1.50±0.12
	2	0±0	0±0	0.17±0.09	0.11±0.08	0.17±0.09	0.67±0.11	1.06±0.13	1.33±0.16	1.61±0.12
SASP (mg/kg)	25	0±0	0±0	0.22±0.10	0.17±0.09	0.22±0.10	0.56±0.12	1.06±0.10	1.17±0.09 ^b	1.44±0.12 ^a

Each value represents the mean±S.E.M. of 17–18 animals. ^aP<0.05, ^bP<0.01 vs control.

method was based on our observation of stool conditions in a pilot study: Grade 0, stool without blood; Grade 1, stool with blood; Grade 2, constant bleeding from the anus. On day 10, the blood leukocyte number was counted by a Coulter counter (Sysmex K-2000; Sysmex Co., Tokyo). The rectums and descending colons (7 cm) were excised promptly. They were opened longitudinally and fixed to be dilated for more than one week. After staining with Carrazi's hematoxylin (Muto Pure Chemicals, Ltd., Tokyo), the area of erosion stained with dark purple was determined by an image analyzer (LA-555; Pierce, Ltd., Osaka). The colons were embedded with paraffin. Paraffin sections were prepared and stained with hematoxylin-eosin. The inflammatory cells infiltrated into the lamina propria and the submucosa of the rectum were counted under a microscope (×640, DMBR; Leica, Heidelberg, Germany). All results are presented as values of the mean±S.E.M. Dunnett's test was used for multiple comparisons.

Two rats from the control group, and one rat from each group given PC-SOD at 0.25, 1 or 2 mg/kg died by the end of the experiment. There was no significant difference between each group in the quantity of 3% DSS ingested (data not shown). Bloody stools in the control group started on day 2 after DSS administration and further worsened by day 10 in 100% of the animals. They were observed between days 3–5 in each group administered with PC-SOD. Treatment with PC-SOD at 0.25, 0.5 and 1 mg/kg significantly reduced the bloody stool score (Table 1). The optimum doses of PC-SOD were 0.5 and 1 mg/kg. Treatment with SASP also significantly reduced the bloody stool score (Table 1). The blood leukocyte count in the control group given 3% DSS increased to approximately six times higher than that of the normal group. Treatment with 1 mg/kg PC-SOD significantly reduced the increase of leukocytes in the blood (Fig. 1), but treatment with SASP did not (Fig. 1). Erosion was observed on the rectum and descending colon. It was found in all animals except two rats from the PC-

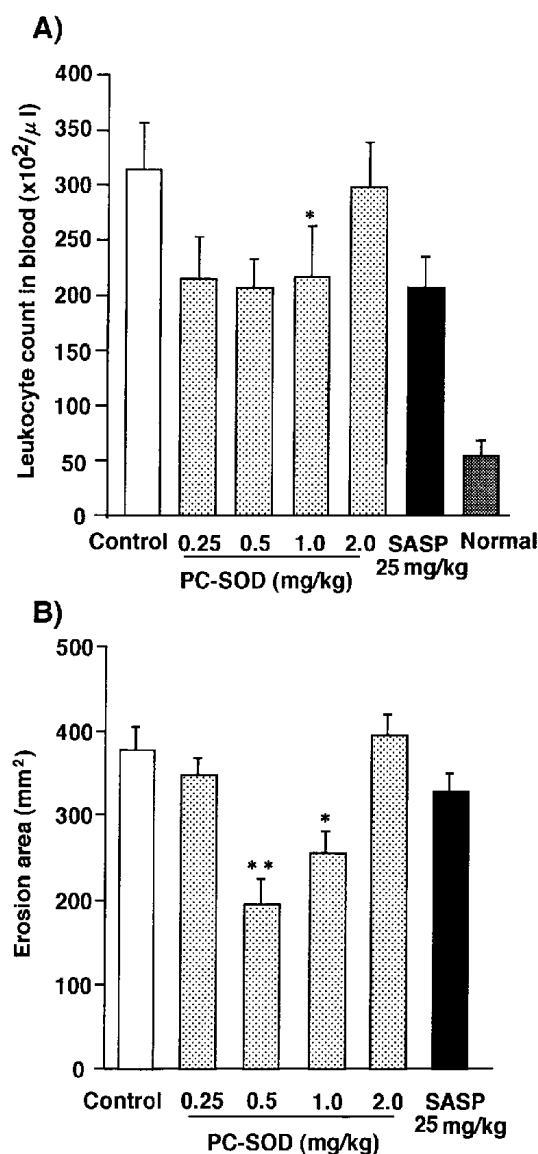


Fig. 1. Effect of PC-SOD on leukocyte count in blood (A) and erosion area on the rectum and descending colon (B). Each value represents the mean±S.E.M. of 16–18 animals. *P<0.05, **P<0.01 vs control.

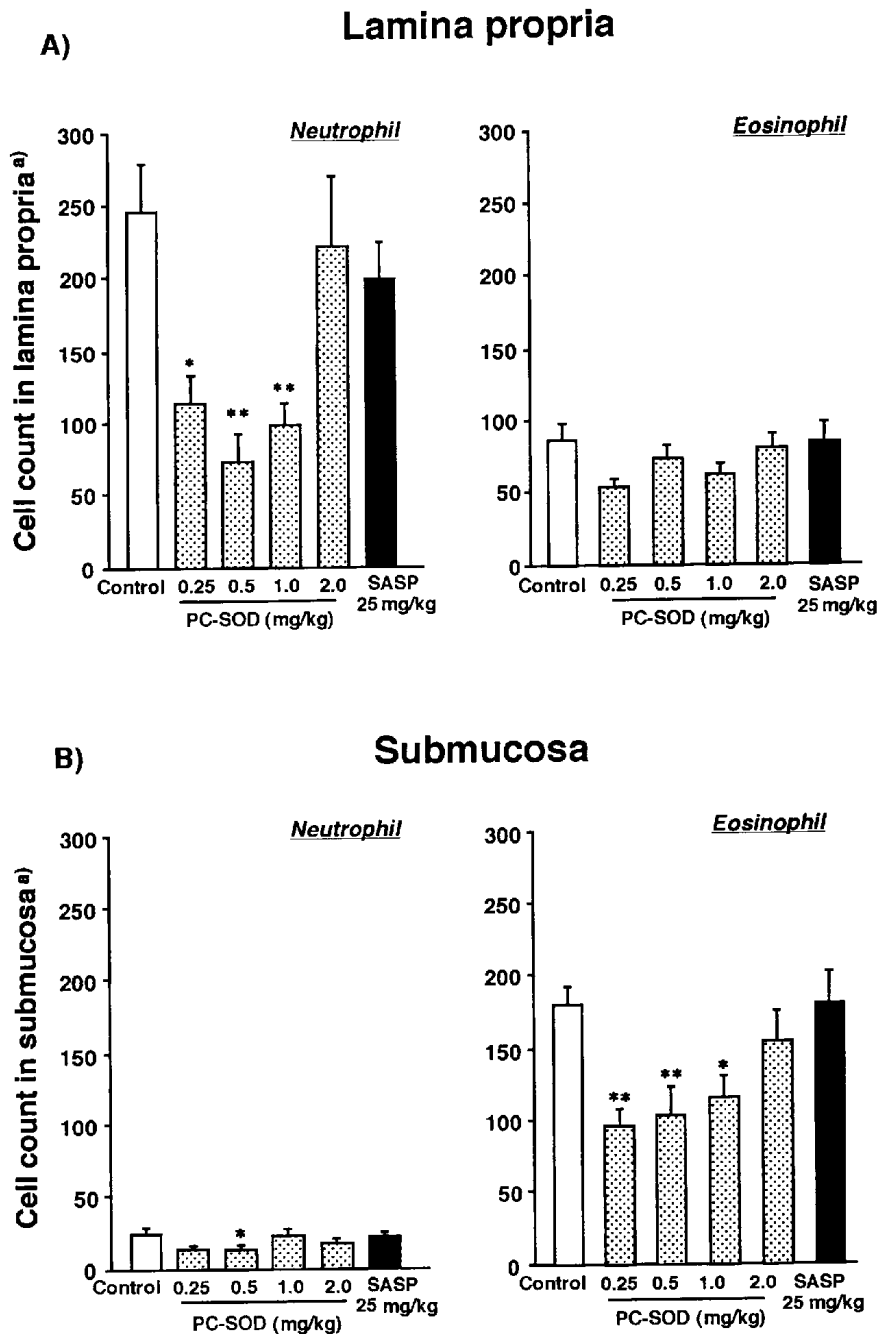


Fig. 2. Effect of PC-SOD on infiltration of inflammatory cells in lamina propria of rectum (A) and submucosa of rectum (B). Each value represents the mean \pm S.E.M. of 17–18 animals. * $P < 0.05$, ** $P < 0.01$ vs control. ^aTotal cell count of nine areas (0.248 mm^2).

SOD (0.5 mg/kg) group and one rat from the PC-SOD (1 mg/kg) group (Fig. 1). Treatment with PC-SOD at 0.5 and 1 mg/kg significantly reduced the erosion area (Fig. 1). However, treatment with SASP did not reduce the erosion area (Fig. 1). In the control group given 3% DSS, a marked inflammation was observed in all specimens. In the submucosa of the rectum, a marked infiltration of

eosinophils was found, while in the lamina propria of the rectum, a marked infiltration of inflammatory cells such as neutrophils, eosinophils and macrophages was observed. In this study, we examined the infiltration of neutrophils and eosinophils by counting these cells in the lamina propria and submucosa. Treatment with PC-SOD at 0.25, 0.5 and 1 mg/kg significantly inhibited infiltra-

tion of neutrophils into the lamina propria and eosinophils into submucosa (Fig. 2). Treatment with SASP did not inhibit infiltration of these cells (Fig. 2). Additionally, no toxic effects were observed when normal rats were treated with 2 mg/kg PC-SOD (data not shown).

No reports have shown that the intravenous administration of free SOD has a therapeutic effect in the DSS induced ulcerative colitis model. This is because free SOD has a short half-life in blood and a low affinity to both endothelial cells and neutrophils (6, 7). In this study, we examined the effect of PC-SOD on this model. PC-SOD suppressed the progression of bloody stools, the formation of erosion and the infiltration of inflammatory cells into the colon. It also reduced the increase of leukocytes in the blood, which is a characteristic of the acute fulminating type of ulcerative colitis. The range of effective doses was between 0.25–1 mg/kg, and the optimum dose was 0.5 mg/kg. SASP, which has an inhibitory action on the generation of O_2^- from neutrophils (10), significantly inhibited the presence of bloody stools. However, the inhibitory effect of SASP appeared at the late phase, and SASP did not suppress the formation of erosion finally. Recently, it was reported that there was a lag time for the appearance of the therapeutic effects of SASP on this model (11). We think that this is the reason why the effects of SASP are weaker than those of PC-SOD. PC-SOD has a longer plasma half-life and higher affinity to both endothelial cells and neutrophils than free SOD (6, 7). PC-SOD reduced endothelial cell injury by neutrophils at lower doses than recombinant human SOD (7). It is thought that PC-SOD scavenges O_2^- efficiently on the surface of the cell membrane, where there is a lack of anti-oxidant enzymes. O_2^- reacts with nitric oxide (NO) to form peroxynitrite, which is a relatively long-lived, strong oxidant (12). Accordingly, in 3%-DSS-induced rat colitis, it is thought that PC-SOD prevents tissue damage by efficiently scavenging O_2^- from inflammatory cells. Therefore, the therapeutic effect of PC-SOD may appear earlier than that of SASP. PC-SOD inhibited cellular infiltration in the colon, particularly that of neutrophils in the lamina propria. Eosinophils migrate in response to interleukin-4 (IL-4) generated from mast cells or T lymphocytes. On the other hand, neutrophils not only migrate in response to interleukin-8 (IL-8), but are also activated to produce more IL-8 in the injured mucosa and thus the mucosa is further damaged. The PC-SOD may inhibit neutrophil infiltration by preventing tissue damage. NO inhibits leukocyte adhesion to the endothelium. O_2^- scavenging by SOD leads to the formation of the long-lived NO. It is also suggests that the inhibitory effect of PC-SOD on cellular infiltration may be partially due to the life extendency of NO. On the other hand, the dose-response curve for PC-SOD showed

a bell-shape pattern, like free SOD (13, 14). SOD produces H_2O_2 in the process of scavenging O_2^- . The H_2O_2 reacts with Fe^{2+} and then produces hydroxyl radical ($OH\cdot$) according to the Fenton reaction (15). The $OH\cdot$ induces severe damage to tissues and cells. In the group receiving the highest dose of PC-SOD (2 mg/kg), it seems that the therapeutic effect of PC-SOD was lost due to the excess production of $OH\cdot$.

In summary, using the 3%-DSS-induced rat colitis model, PC-SOD decreased the presence of bloody stools, formation of erosion and inflammatory cell infiltration in the large bowel. It also suppressed the increase of leukocytes in the blood. The major mechanism of the therapeutic effect of PC-SOD is the prevention of tissue damage by efficiently scavenging oxygen radicals. Therefore, PC-SOD may have therapeutic potential for the treatment of ulcerative colitis.

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