Original

Effects of Pretreatment with PSK, a Protein-Bound Polysaccharide, on *Escherichia Coli* Infection in Mice

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Abstract: Only 17% of mice inoculated with lethal doses of *Escherichia coli* survived for 5 days after the challenge. Significant antibacterial activity of PSK, a protein-bound polysaccharide, was induced when the mice were pretreated with 40 mg/kg of PSK by an intraperitoneal, subcutaneous or intramuscular route, but not by an intravenous route, 48 hr before the *E. coli* inoculation. Repeated oral administration of PSK for 2 weeks (20 mg/kg, 3 times per week) before *E. coli* inoculation was also effective. Intraperitoneal administration of PSK was most potent, inducing significant polymorphonuclear cell accumulation and generation of luminol-dependent chemiluminescence by peritoneal exudate cells, with a peak 24 hr after administration of the PSK.

Key words: PSK, *E. coli* infection, antibacterial activity, polymorphonuclear cells, chemiluminescence

Introduction

A protein-bound polysaccharide, PSK, extracted from the mycelium of *Coriolus versicolor* (Fr.) Quel, has been recognized for its host-mediated antitumor activity.¹⁾ However, there are few reports of PSK effects on experimental infections in mice.^{2,3)} To better understand the induction of antibacterial activity, we investigated the effects of PSK administered by various routes on *Escherichia coli* infection in mice, and the kinetics of the accumulation and activation of the peritoneal exudate cell populations after PSK administration.

Materials and Methods

Materials. PSK prepared from the mycelium of a CM-101 strain of *Coriolus versicolor*¹⁾ was kindly provided by Kureha Chem. Ind., Ltd., Tokyo.

Mice. Female ICR mice (5 weeks old, 23–25 g) were obtained from Sankyo Labo. Service Co., Tokyo. The mice were used at 6–7 weeks of age.

Antibacterial activity. Each mouse was given a single injection of saline or PSK by one of the indicated administration routes, 48 hr before inoculation with *E. coli* (GN2411) (4×10^6 cells). Survival rates of the mice were determined 5 days after *E. coli* challenge unless otherwise stated.^{3,4)}

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Peritoneal exudate cell collection. Mice were killed by decapitation and peritoneal fluid was withdrawn through a 22-gauge needle inserted into the cavity. The content of total polymorphonuclear cells (PMN) in the fluid was determined by the following formula: PMN content = $A \times B$; where A was the total cell number in the peritoneal fluid, determined with a hemocytometer, and B was the percent of PMN in all of the cells, assessed on Cytospin-slides (Shandon Southern Products Ltd., Astmoor, England) stained with May-Grunwald-Giemsa (Merck, Darmstadt, West Germany).

Chemiluminescence assay. The peritoneal exudate cells (1×10^6) were mixed with 1.25 mg of opsonized zymosan A (Sigma, St. Louis, MO, USA) and 20 μ g of luminol (Tokyo Kasei Kogyo Co., Ltd., Tokyo) in 0.6 ml of Eagle's MEM medium without phenol red (Nissui Pharm. Co., Ltd., Tokyo). Light emission was measured at 37°C immediately, and at 3 minute intervals over a 15 minute period, with the aid of an Aloka BLR-102 luminescence reader (Aloka Ltd., Tokyo) equipped with a controller. The chemiluminescence values were given as peak cpm instead of integral cpm.^{4,5)}

Results and Discussion

When mice were inoculated with about 4×10^6 cells of *E. coli*, only 17% survived after 1 day (Fig. 1). However, if pretreated intraperitoneally with 40 mg/kg PSK 2 days before the *E. coli* challenge, the survival ratio increased to 80%. Since the survival ratio of the pretreated mice became stable 5 days after the *E. coli* challenge, the antibacterial activity was assayed at that time. The optimal concentration of PSK was 40 mg/kg (Figs. 1 and 2); doses greater than this were less effective. The induction of antibacterial activity also depended on the treatment time (Fig. 2). The effect of PSK became detectable 4 hr after treatment, reached a maximum after 1–3 days and then declined almost to the control level.

Fig. 1.

Fig. 2.

Fig. 1. Dose-dependent inhibition of *E. coli* infection by PSK administration.

Mice (10–12/group) were intraperitoneally administered 0 (□), 2 (△), 8 (○), 40 (●) or 80 (◇) mg/kg PSK, 48 hr before *E. coli* challenge. Survival rates were determined at the indicated days after *E. coli* challenge.

Fig. 2. Dependence of inhibition of *E. coli* infection on time of treatment with PSK.

Mice (10–12/group) were intraperitoneally administered 0 (□), 0.4 (▽), 2 (△), 8 (○) or 40 (●) mg/kg PSK for the indicated periods before *E. coli* challenge. Survival rate was determined 5 days after *E. coli* challenge.

Table 1 shows that significant antibacterial activity of PSK was induced when the mice were administered 40 mg/kg PSK by either an intraperitoneal, subcutaneous, or intramuscular route (Exp. I), but not by an intravenous route (Exp. II), 48 hr before *E. coli* inoculation. The most potent effect was produced by an intraperitoneal administration of PSK. It should be noted that repeated oral administration of PSK for 2 weeks (20 mg/kg, 3 times per week) before *E. coli* inoculation was effective (Table 1, Exp. III).

Intraperitoneal administration of PSK induced significant accumulation of polymorphonuclear cells (PMN) (Fig. 3A) and stimulated the generation of luminol-dependent chemiluminescence (LDCL) by peritoneal exudate cells (Fig. 3B). Both the number of PMN and the LDCL generation of the peritoneal exudate cells increased to a peak at 24 hr after PSK

Table 1. Effect of administration routes on PSK inhibition of *E. coli* infection.

Dose (mg/kg)	Administration route	No. of survivors
Exp. I:		
40×1	i.p.	10/10
40×1	s.c.	8/10
40×1	i.m.	8/10
0×1 (control)	—	2/12
Exp. II:		
40×1	i.v.	0/10
0×1 (control)	—	0/12
Exp. III:		
20×6	p.o.	8/10
0×6 (control)	—	3/12

Mice (10–12/group) were administered one dose of saline (control) or PSK by the indicated routes 48 hr before *E. coli* inoculation, except for oral administration (20 mg/kg×6). Survival ratio was determined 5 days after *E. coli* inoculation.

Fig. 3. Time course of PMN accumulation in the peritoneal cavity (A), and LDCL generation by peritoneal exudate cells (B) after intraperitoneal administration of 0 (○) or 40 (●) mg/kg PSK.

Each value is mean from 2 mice. S.D. < 20%.

administration, and then declined almost to the control level at 48 hr.

We observed the lethal effect of *E. coli* infection within 4 days after challenge, and intra-peritoneal administration of PSK significantly reduced this lethal effect (Fig. 1). Since PSK induced rapid accumulation of PMN, which had higher LDCL generating activity (Fig. 3), it is highly probable that PSK-induced PMN might be responsible for the rapid disappearance of *E. coli*, especially in the early stages (0–48 hr) after PSK administration. A potential role of PMN was further supported by our finding that intravenous administration of 40 mg/kg PSK failed to inhibit *E. coli* infection in mice (Table 1, Exp. II) or to stimulate LDCL generation by the peritoneal exudate cells (9,400 cpm/mouse (PSK treated) vs 19,700 cpm/mouse (control) (data not shown).

The PSK was a mixture of at least four subfractions, each with distinct molecular weight⁽⁶⁾ and isoelectric point.⁽⁷⁾ We previously reported that the highest molecular weight PSK subfraction (MW>200 kD), which induced more potent antibacterial activity in mice than the other lower molecular weight subfractions, also more greatly stimulated LDCL generation by peritoneal exudate cells.⁽³⁾ Further study is necessary to elucidate the mechanism of antimicrobial activity induction by these PSK fractions.

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