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The effects of delayed treatment with sialyl Lewis X against lipopolysaccharide-induced acute lung injury in rabbits

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Abstract

The therapeutic effects of a selectin inhibitor against lipopolysaccharide-induced acute lung injury were studied in rabbits by using sialyl Lewis X-oligosaccharide. Lipopolysaccharide-induced acute lung injury, as characterized by an impairment of pulmonary gas exchange, clinically resembles that of the acute respiratory distress syndrome. Delayed treatments with sialyl Lewis X-oligosaccharide (55 mg kg^{-1} i.v. bolus injection 0.5, 1 or 2 h after lipopolysaccharide administration + 36 mg kg^{-1} h^{-1} i.v. infusion for 5.5, 5 or 4 h, respectively) prevented the lipopolysaccharide-induced impairments in pulmonary gas exchange, as well as the accumulation of polymorphonuclear leukocytes in the lung tissue. In contrast, this agent had no significant effects on lipopolysaccharide-induced systemic hypotension, the decrease in the number of circulating white blood cells and platelets or the decline in blood pH.

This is the first demonstration that sialyl Lewis X-oligosaccharide is effective against the impairments in pulmonary gas exchange even if administered 0.5, 1 or 2 h following the lipopolysaccharide injection.

Keywords: Selectin; Sialyl Lewis X; Acute lung injury; Lipopolysaccharide

1. Introduction

The selectin family is a group of cell adhesion molecules which includes E-, L- and P-selectins. These molecules are involved in the first step of inflammatory processes, the adherence of polymorphonuclear leukocytes to activated vascular endothelial cells (Granger and Kubes, 1994). The sialyl Lewis X-oligosaccharide motif is a carbohydrate ligand for all three selectins (Bevilacqua and Nelson, 1993) and has previously been used as a selectin inhibitor. We and others have demonstrated that sialyl Lewis X-oligosaccharide attenuates ischemia/reperfusion-induced myocardial necrosis in feline (Buerke et al., 1994), canine (Lefer et al., 1994; Flynn et al., 1996), rat (Tojo et al., 1996) and rabbit (Yamada et al., 1998) models, as well as ischemia/reperfusion injury in the rabbit ear (Han et al., 1995). In addition, we have recently demonstrated that this inhibitor attenuates neutrophil-dependent myocardial dysfunction in the isolated rat heart (Ohnishi et al., 1999).

Sialyl Lewis X-oligosaccharide has also been used in various acute lung injury models. Ridings et al. (1997) showed that prophylactic treatment with sialyl Lewis X-oligosaccharide attenuated pulmonary dysfunction in a porcine sepsis model. Recently, we have demonstrated that prophylactic treatment with sialyl Lewis X-oligosaccharide and anti-P-selectin antibody, respectively prevented lipopolysaccharide-induced impairments in pulmonary gas exchange, as well as the infiltration of polymorphonuclear leukocytes into the lung of rabbits (Hayashi et al., 1999). It should be noted, however, that all studies using sialyl Lewis X-oligosaccharide have been conducted in order to examine its prophylactic effects except for a study on ischemia/reperfusion injury in the rabbit ear (Han et al., 1995). In the present study, we examined for the first time, whether the delayed treatment with sialyl Lewis X-oligosaccharide could improve the ability of pulmonary gas exchange and protect it from lipopolysaccharide-induced acute lung injury.

2. Materials and methods

2.1. Materials

Male New Zealand White rabbits (SPF, 2.0–2.5 kg) were purchased from Kitayama Labes (Tokyo, Japan).

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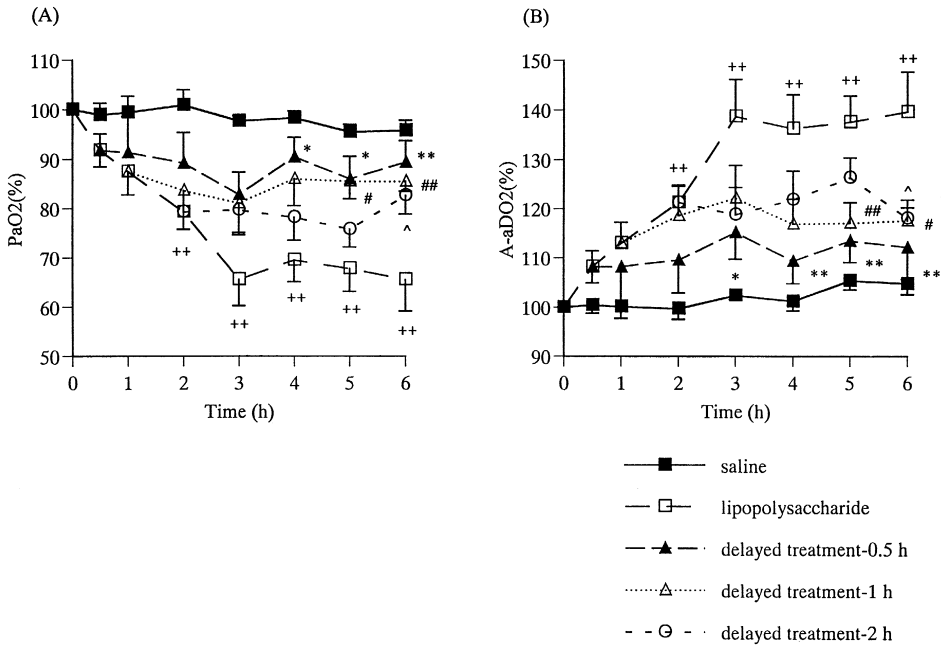


Fig. 1. Effects of sialyl Lewis X-oligosaccharide on PaO_2 and A-aDO_2 in lipopolysaccharide-induced lung injury. Lipopolysaccharide (0.3 mg kg^{-1}) was injected intravenously and PaO_2 (A) was monitored for 30 min and then every hour after lipopolysaccharide injection. A-aDO_2 (B) was calculated as described in Section 2. Delayed treatment − 0.5 h group received sialyl Lewis X-oligosaccharide (55 mg kg^{-1} i.v. bolus injection 0.5 h after lipopolysaccharide administration + 36 mg kg^{-1} h^{-1} i.v. infusion for 5.5 h). Delayed treatment − 1 h group received sialyl Lewis X-oligosaccharide (55 mg kg^{-1} i.v. bolus injection 1 h after lipopolysaccharide administration + 36 mg kg^{-1} h^{-1} i.v. infusion for 5 h). Delayed treatment − 2 h group received sialyl Lewis X-oligosaccharide (55 mg kg^{-1} i.v. bolus injection 2 h after lipopolysaccharide administration + 36 mg kg^{-1} h^{-1} i.v. infusion for 4 h). Each value represents the mean ± S.E.M. of eight determinations. Statistical significance: $^{++}P < 0.01$ for lipopolysaccharide group vs. saline group, $^{**}P < 0.01$ for delayed treatment − 0.5 h group vs. lipopolysaccharide group, $^{*}P < 0.05$ for delayed treatment − 0.5 h group vs. lipopolysaccharide group, $^{\#\#}P < 0.01$ for delayed treatment − 1 h group vs. lipopolysaccharide group, $^{\#}P < 0.05$ for delayed treatment − 1 h group vs. lipopolysaccharide group, $^{\wedge}P < 0.05$ for delayed treatment − 2 h group vs. lipopolysaccharide group.

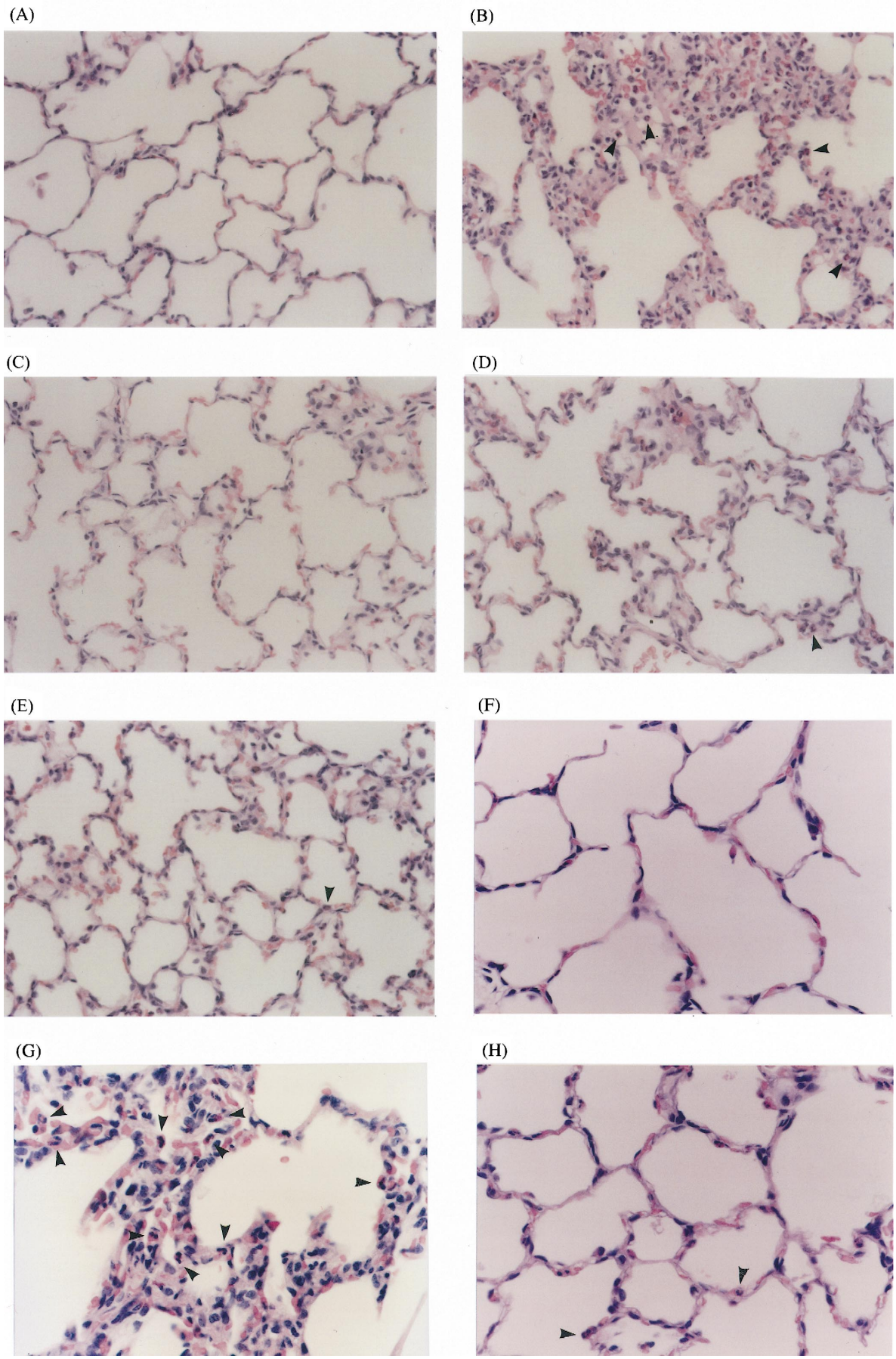
Prior to the experiments, they were housed for a minimum of 1 week in a quarantine room with a 12 h:12 h daily light/dark cycle. Lipopolysaccharide (*Salmonella minnesota*) and gallamine triethiodide were purchased from Sigma-Aldrich Japan (Tokyo, Japan) and diluted with 0.3 and 10 mg ml^{-1} saline, respectively. Sialyl Lewis X-oligosaccharide is an oligosaccharide analog, which was prepared by combined chemical and enzymatic synthesis (Ichikawa et al., 1992) and was kindly provided by Cytel (San Diego, CA, USA). Other reagents used were of the highest grade commercially available.

2.2. Animal preparation

All procedures related to the use of animals in these studies were reviewed and approved by the Institutional Animal Care and Use Committee at Sumitomo Pharmaceuticals Research Center (Osaka, Japan). The experimental procedures were essentially based on those previously reported (Hayashi et al., 1999). In brief, animals were anesthetized by the administration of sodium pentobarbital (40 mg kg^{-1}) through the ear veins. After tracheae were cannulated with silicon tubes, animals were ventilated (concentration of O_2 in inspired gas (FiO_2); 0.52, tidal volume; 30 ml, respiratory rate; 30 rpm) using volume-controlled respirators (SN-480-5, Sinano, Tokyo, Japan). To arrest spontaneous breathing, animals were injected intramuscularly with 12.5 mg per head of gallamine triethiodide every 2 h. A catheter was placed in the right common carotid artery for monitoring mean arterial blood pressure and for sampling blood, and a silicon tube was introduced into the right femoral vein for the administration of lipopolysaccharide and sialyl Lewis X-oligosaccharide.

Approximately 20–30 min later, when the arterial blood oxygen tension (PaO_2) and arterial blood carbon dioxide tension ($PaCO_2$) had stabilized, lipopolysaccharide (0.3

Fig. 2. Histopathologic examination of rabbit lungs of lipopolysaccharide-induced lung injury (hematoxylin and eosin). Lipopolysaccharide (0.3 mg kg^{-1}) was injected intravenously into male New Zealand White rabbits. Four lung tissues per group collected 6 h after lipopolysaccharide injection were fixed with 10% formalin. Tissue slices (one slice per lung tissue) were stained with hematoxylin and eosin. The typical micrographs of lung tissues are shown (magnification: ×400): (A) saline group, (B) lipopolysaccharide group, (C) delayed treatment − 0.5 h group, (D) delayed treatment − 1 h group, (E) delayed treatment − 2 h group, (magnification: ×600), (F) saline group, (G) lipopolysaccharide group, (H) delayed treatment − 0.5 h group. The infiltrating polymorphonuclear leukocytes are indicated by the arrowheads.


(A)
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mg kg^{-1}) was administered intravenously. The experimental conditions were comparable to those reported by Nishina et al. (1997). The initial level of PaO_2 was approximately 150 mm Hg. Arterial blood was collected for 30 min, then every hour after, lipopolysaccharide injection was subjected to blood gas analysis, using a blood gas analyzer (Type 273, Chiron, Boston, MA, USA), and the numbers of white blood cells and platelets were counted using an automated blood cell counter (Sysmex F-800, Sysmex, Kobe, Japan). The mean arterial blood pressure and heart rate via the right common carotid artery were amplified and monitored using a pressure transducer (P10EZ-1, Nihon Koden, Tokyo, Japan) connected to an electric recorder (WT-685, Nihon Koden, Tokyo, Japan). Animals were killed with an overdose of sodium pentobarbital 6 h after lipopolysaccharide injection.

The difference between alveolar and arterial blood oxygen tension (A-aDO_2) was calculated according to the formula below (Mellemgarrd, 1966):

$$\begin{aligned}\text{A-a}DO_2(\text{mm Hg}) &= \text{alveolar } O_2 \text{ tension} - PaO_2\\ &= \left[FiO_2 \times (760-47) - (PaCO_2)\right.\\ &\quad \left.\times\{FiO_2 + (1-FiO_2)/0.8\}\right] - PaO_2\\ &= (0.52 \times 713 - 1.12 \times PaCO_2) - PaO_2.\end{aligned}$$

2.3. Experimental protocols

Animals were assigned randomly to five groups. In the saline group ($n = 8$), animals were intravenously injected with saline (1 ml kg^{-1}), then infused for over 6 h with saline (1 ml kg^{-1} h^{-1}). In the lipopolysaccharide group ($n = 8$), animals were intravenously injected with lipopolysaccharide (0.3 mg kg^{-1}, 1 ml kg^{-1}), then infused for over 6 h with saline (1 ml kg^{-1} h^{-1}). In the sialyl Lewis X-oligosaccharide (delayed treatment − 0.5 h) group ($n = 8$), animals were intravenously injected with sialyl Lewis X-oligosaccharide (55 mg kg^{-1}, 1 ml kg^{-1}) 0.5 h after lipopolysaccharide injection. Sialyl Lewis X-oligosaccharide (36 mg kg^{-1} h^{-1}, 1 ml kg^{-1} h^{-1}) was then infused for over 5.5 h. In the sialyl Lewis X-oligosaccharide (delayed treatment − 1 h) group ($n = 8$), animals were intravenously injected with sialyl Lewis X-oligosaccharide (55 mg kg^{-1}, 1 ml kg^{-1}) 1 h after lipopolysaccharide injection. The infusion of sialyl Lewis X-oligosaccharide (36 mg kg^{-1} h^{-1}, 1 ml kg^{-1} h^{-1}) followed for over 5 h. In the sialyl Lewis X-oligosaccharide (delayed treatment − 2 h) group ($n = 8$), animals were intravenously injected with sialyl Lewis X-oligosaccharide (55 mg kg^{-1}, 1 ml kg^{-1}) 2 h after lipopolysaccharide injection. Then sialyl Lewis X-oligosaccharide (36 mg kg^{-1} h^{-1}, 1 ml kg^{-1} h^{-1}) was infused for over 4 h.

2.4. Histopathologic examination

Immediately after the rabbits were killed, all lobes of the left lung were fixed by the instillation of 10% formaldehyde solution through the left bronchus at 50 cm H_2O. The specimens were embedded in paraffin wax and then stained with hematoxylin-eosin. The number of polymorphonuclear leukocytes, identified by the typical polymorphonuclear appearance, was counted microscopically in the field (magnification: ×600) of tissue sections. Three fields per tissue section were used for quantitative analysis by a blinded observer.

2.5. Statistical analysis

The significance of the differences between the two groups, such as the saline and lipopolysaccharide groups, was analyzed by using the Student's *t*-test. The significance of differences between more than two groups, on the other hand, was analyzed by using Dunnett's *t*-test with the SAS program (SAS Institute, Cary, NC, USA). Probabilities less than 0.05 were considered statistically significant.

3. Results

In the saline group, PaO_2 did not change during the experiment. The PaO_2 in the lipopolysaccharide group declined rapidly until 3 h after the lipopolysaccharide injection. The levels declined by approximately 20% at 2 h and by approximately 35% at 3 h as compared with the level at 0 h. The PaO_2 level of the lipopolysaccharide group was significantly different from that in the saline group from 2 h after lipopolysaccharide injection onwards

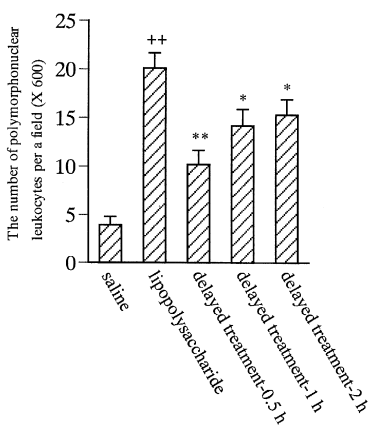

Fig. 3. Effects of sialyl Lewis X-oligosaccharide on the number of infiltrated polymorphonuclear leukocytes in lung. Lipopolysaccharide (0.3 mg kg^{-1}) was injected intravenously. Four lung tissues per group collected 6 h after lipopolysaccharide injection were fixed with 10% formalin. Tissue slices (one slice per lung tissue) were stained with hematoxylin and eosin. The number of polymorphonuclear leukocytes (magnification: ×600) was counted as described in Section 2. Each value represents the mean ± S.E.M. of 12 determinations. Statistical significance: $^{++}P < 0.01$ for lipopolysaccharide group vs. saline group, $^{**}P < 0.01$ for delayed treatment group vs. lipopolysaccharide group, $^{*}P < 0.05$ for delayed treatment group vs. lipopolysaccharide group.

Table 1
Effect of sialyl Lewis X-oligosaccharide on various parameters
Delayed treatment − 0.5 h group received sialyl Lewis X oligosaccharide 55 mg kg^{-1} i.v. bolus 0.5 h after lipopolysaccharide injection and then sialyl Lewis X-oligosaccharide (36 mg kg^{-1} h^{-1} i.v., 1 ml kg^{-1} h^{-1}) for 5.5 h. Delayed treatment − 1 h group received sialyl Lewis X-oligosaccharide 55 mg kg^{-1} i.v. bolus 1 h after lipopolysaccharide injection and then sialyl Lewis X-oligosaccharide (36 mg kg^{-1} h^{-1} i.v., 1 ml kg^{-1} h^{-1}) for 5 h. Delayed treatment − 2 h group received sialyl Lewis X-oligosaccharide 55 mg kg^{-1} i.v. bolus 2 h after lipopolysaccharide injection and then sialyl Lewis X-oligosaccharide (36 mg kg^{-1} h^{-1} i.v., 1 ml kg^{-1} h^{-1}) for 4 h. Each point represents the mean ± S.E.M..

Parameters	Treatment group	Hours after lipopolysaccharide injection							
		0 h	0.5 h	1 h	2 h	3 h	4 h	5 h	6 h
Mean arterial blood pressure (% of the value at 0 h)	saline ($n=8$)	100	99.6 ± 1.3	94.2 ± 1.8	93.4 ± 4.5	96.4 ± 1.9	91.6 ± 4.8	90.3 ± 3.7	83.9 ± 7.0
	lipopolysaccharide ($n=8$)	100	94.7 ± 1.7	88.1 ± 1.5**	85.4 ± 3.2	79.7 ± 7.0**	72.0 ± 6.8**	75.3 ± 2.3*	66.9 ± 6.7
	delayed treatment − 0.5 h ($n=8$)			81.7 ± 4.3	79.3 ± 3.8	79.5 ± 7.3	81.1 ± 7.5	78.6 ± 9.8	73.1 ± 9.4
	delayed treatment − 1 h ($n=8$)				84.5 ± 2.7	81.6 ± 5.3	82.3 ± 4.9	83.6 ± 4.5	80.0 ± 4.4
	delayed treatment − 2 h ($n=8$)					87.0 ± 4.1	86.6 ± 5.8	81.9 ± 7.1	77.7 ± 6.3
Number of peripheral white blood cells (% of the value at 0 h)	saline ($n=8$)	100	91.7 ± 7.7	83.4 ± 13	51.0 ± 4.2	64.6 ± 17	87.3 ± 18	90.4 ± 18	108 ± 17
	lipopolysaccharide ($n=8$)	100	90.7 ± 2.5	81.6 ± 2.6	43.6 ± 2.6	34.0 ± 3.7	34.3 ± 5.2**	41.6 ± 6.6**	49.1 ± 6.5*
	delayed treatment − 0.5 h ($n=8$)			74.9 ± 3.7	44.2 ± 4.9	36.1 ± 3.7	40.5 ± 4.7	51.7 ± 5.5	50.2 ± 4.3
	delayed treatment − 1 h ($n=8$)				44.6 ± 3.1	34.2 ± 3.6	38.5 ± 4.3	49.5 ± 4.5	50.3 ± 5.0
	delayed treatment − 2 h ($n=8$)					33.3 ± 3.3	38.5 ± 3.8	44.7 ± 6.8	49.7 ± 5.9
Number of platelets (% of the value at 0 h)	saline ($n=8$)	100	98.3 ± 5.3	98.5 ± 3.9	87.1 ± 6.9	90.4 ± 5.9	86.9 ± 4.3	84.5 ± 3.6	74.1 ± 6.3
	lipopolysaccharide ($n=8$)	100	47.0 ± 2.9**	63.5 ± 2.8**	69.1 ± 3.3*	68.4 ± 4.9*	61.6 ± 3.7**	61.0 ± 4.1**	54.9 ± 5.9**
	delayed treatment − 0.5 h ($n=8$)			69.8 ± 14	72.4 ± 12	69.6 ± 12	60.7 ± 7.9	62.8 ± 12	58.0 ± 11
	delayed treatment − 1 h ($n=8$)				65.4 ± 3.6	65.1 ± 3.6	62.6 ± 4.2	60.3 ± 4.5	57.3 ± 4.0
	delayed treatment − 2 h ($n=8$)					70.1 ± 4.4	65.6 ± 4.0	60.8 ± 4.5	58.5 ± 4.5
Blood pH	saline ($n=8$)	7.49 ± 0.01	7.46 ± 0.01	7.45 ± 0.02	7.45 ± 0.02	7.44 ± 0.01	7.43 ± 0.02	7.44 ± 0.01	7.43 ± 0.01
	lipopolysaccharide ($n=8$)	7.50 ± 0.01	7.45 ± 0.01	7.44 ± 0.01	7.41 ± 0.03*	7.38 ± 0.02*	7.39 ± 0.03	7.39 ± 0.03	7.38 ± 0.04
	delayed treatment − 0.5 h ($n=8$)			7.40 ± 0.03	7.40 ± 0.03	7.37 ± 0.04	7.38 ± 0.04	7.36 ± 0.05	7.36 ± 0.05
	delayed treatment − 1 h ($n=8$)				7.41 ± 0.02	7.42 ± 0.01	7.44 ± 0.01	7.43 ± 0.02	7.44 ± 0.02
	delayed treatment − 2 h ($n=8$)					7.41 ± 0.02	7.43 ± 0.02	7.44 ± 0.02	7.41 ± 0.04
Arterial CO_2 tension ($PaCO_2$) (% of the value at 0 h)	saline ($n=8$)	100	105 ± 4.9	105 ± 7.7	101 ± 6.7	101 ± 5.3	107 ± 11	96.3 ± 4.3	96.5 ± 4.5
	lipopolysaccharide ($n=8$)	100	111 ± 3.7	108 ± 7.3	107 ± 7.4	99.1 ± 5.1	89.8 ± 3.7	91.5 ± 7.7	95.3 ± 3.1
	delayed treatment − 0.5 h ($n=8$)			115 ± 5.8	111 ± 7.4	109 ± 3.9	102 ± 2.7	98.5 ± 2.7	93.1 ± 11
	delayed treatment − 1 h ($n=8$)				111 ± 17	99.2 ± 9.6	97.8 ± 7.3	95.0 ± 7.9	94.0 ± 6.8
	delayed treatment − 2 h ($n=8$)					115 ± 4.7	104 ± 5.5	97.2 ± 3.8	94.9 ± 2.5

** Statistical significance: $P < 0.01$ vs. saline group by Student's *t*-test
* Statistical significance: $P < 0.05$ vs. saline group by Student's *t*-test.

(Fig. 1A). In contrast, the delayed treatments with sialyl Lewis X-oligosaccharide prevented further declines in the PaO_2 level. The PaO_2 level in the sialyl Lewis X-oligosaccharide (delayed treatment −0.5, −1, and −2 h) groups was significantly higher than that in the lipopolysaccharide group, from 4, 5, and 6 h, respectively, after the lipopolysaccharide injection (Fig. 1A).

Similar results were obtained for the improvement of A-aDO_2 (Fig. 1B). The A-aDO_2 level in the lipopolysaccharide group increased markedly after the lipopolysaccharide injection and became significantly different from that in the saline group from 2 h after lipopolysaccharide injection onwards. Delayed treatment with sialyl Lewis X-oligosaccharide (delayed treatment −0.5, −1, and −2 h) inhibited further increase of the A-aDO_2 levels, and the levels were significant lower than that in the lipopolysaccharide group, from 4, 5, and 6 h, respectively, after lipopolysaccharide injection onwards (Fig. 1B).

Histopathologic examinations of the lungs showed lipopolysaccharide-induced inflammation, such as increased thickness of the alveolar wall and hemorrhage (Fig. 2B and G). In addition, the number of polymorphonuclear leukocytes detected in the lung tissue of the lipopolysaccharide group was significantly higher than that in the saline group (Fig. 3). In contrast, the delayed treatments with sialyl Lewis X-oligosaccharide (delayed treatment −0.5, −1, and −2 h) attenuated those inflammatory profiles (Fig. 2 C–E and H) and significantly decreased the number of polymorphonuclear leukocytes in the lung as compared with the lipopolysaccharide group (Fig. 3).

The mean arterial blood pressure, the number of peripheral white blood cells and platelets, and the blood pH decreased in the lipopolysaccharide group as compared with the saline group. The differences between the saline and lipopolysaccharide group in those parameters were transiently significant. The $PaCO_2$ level transiently increased in the lipopolysaccharide group as compared with the saline group. However, three treatments of sialyl Lewis X-oligosaccharide caused no significant effects on these parameters (Table 1).

4. Discussion

Acute lung injury, which is characterized by pulmonary inflammation, which increased both the permeability edema and the lung compliance, is associated with various diseases including sepsis, acute pancreatitis, burns, and trauma. In sepsis and acute lung injury, it is known that the accumulation of polymorphonuclear leukocytes plays a detrimental role in the development of an acute inflammation (Tate and Repine, 1983; Artigas et al., 1998). In an animal model of sepsis, for example, the rapid sequestration of polymorphonuclear leukocytes into the lung was demonstrated using radiolabeled polymorphonuclear leukocytes (Hangen et al., 1990). Therefore, the blockade of the adhesion of polymorphonuclear leukocytes to pulmonary endothelial cells may provide a novel approach to the treatment of the acute lung injury.

One of the many molecules involved in the adhesion between polymorphonuclear leukocytes and endothelial cells is selectins. In many animal studies, selectin inhibitors have been proved to protect against sepsis with acute lung injury. For example, the administration of sialyl Lewis X-oligosaccharide and anti-P-selectin antibody protected the rats against immune complex-induced acute lung injury (Mulligan et al., 1993; Bless et al., 1998). Recently, we have demonstrated in a rabbit model that lipopolysaccharide-induced pulmonary dysfunction was ameliorated by sialyl Lewis X-oligosaccharide and anti-P-selectin antibody (Hayashi et al., 1999). In addition, we have also observed by histopathological examinations that the number of infiltrating polymorphonuclear leukocytes was increased in the lung interstitial regions in lipopolysaccharide-treated rabbits, but was reduced in rabbits administered with sialyl Lewis X-oligosaccharide prior to the lipopolysaccharide injection. In that study, however, we only examined the effects of prophylactic treatment with the selectin inhibitors against acute lung injury, and therefore, the effects of delayed treatment with the inhibitors remained to be determined. Examination of the effect of delayed treatment would provide useful information on the therapeutic efficacy in clinical settings.

The present study demonstrated that the lipopolysaccharide-induced decrease in PaO_2 is associated with the accumulation of polymorphonuclear leukocytes in the lung and that the number of polymorphonuclear leukocytes in the lung was significantly higher in the lipopolysaccharide group than the saline group. It should be noted, however, that we detected no elevation of the myeloperoxidase activity in the lipopolysaccharide group (data not shown). This is consistent with our previous observation in the similar experimental condition (Hayashi et al., 1999). The administration of sialyl Lewis X-oligosaccharide 0.5, 1 or 2 h after lipopolysaccharide injection protected against the dysfunction of oxygenation (Fig. 1). In addition, the histopathological examinations clearly demonstrated that the delayed treatments with sialyl Lewis X-oligosaccharide significantly inhibited the histological impairments (Fig. 2), as well as the accumulation of polymorphonuclear leukocytes in lung tissue (Fig. 3). Although we did not examine the increase of lung vascular permeability in lipopolysaccharide-induced acute lung injury, we believe that it is very likely that vascular permeability increases in our lipopolysaccharide-treated rabbit lungs in our study. Various studies have previously demonstrated that lipopolysaccharide-induced acute lung injury were well associated with the increase of lung vascular permeability. These studies demonstrated that lipopolysaccharide induced the increase of albumin in bronchoalveolar lavage fluid or lung wet/dry weight ratio in rabbits (Nishina et al., 1997) and in pigs (Hasegawa et al., 1997), and the increase of

protein in bronchoalveolar lavage fluid in pigs (Ridings et al., 1997). In addition, Ridings et al., (1997) demonstrated that sialyl Lewis X inhibited lipopolysaccharide-induced increase of protein content in bronchoalveolar lavage fluid in porcine. Based on these series of observations, we assume that the delayed treatment with sialyl Lewis X also inhibits the lipopolysaccharide-induced increase of permeability in our rabbit model. To our knowledge, this is the first report that demonstrates a protective effect of delayed treatment with sialyl Lewis X-oligosaccharide against acute lung injury. Our results are consistent with those of a previous work that reported the efficacy of sialyl Lewis X-oligosaccharide against ischemia/reperfusion injury of ear in rabbits (Han et al., 1995). In contrast, sialyl Lewis X-oligosaccharide had no effect on the lipopolysaccharide-induced decrease in the mean arterial blood pressure, blood pH, number of peripheral white blood cells or platelets, and increase in the $PaCO_2$ level (Table 1), as previously reported by us in rabbits (Hayashi et al., 1999) and pigs (Ridings et al., 1997).

The most important findings of the present study are that the delayed administration of sialyl Lewis X-oligosaccharide prevented the further progression of impairments in pulmonary gas exchange caused by the lipopolysaccharide and that the treatment did not lead to a full recovery to the control level. For example, when sialyl Lewis X-oligosaccharide was administered 0.5 h after lipopolysaccharide injection, the decreased level of PaO_2 was maintained until 6 h. The PaO_2 level, however, did not return to the initial 0 h level. Similar results were obtained when the inhibitor was administered 1 and 2 h after lipopolysaccharide injection, respectively.

The reduced level of PaO_2 seen at 0.5 h was probably caused by the pre-existing population of polymorphonuclear leukocytes that had infiltrated in lung tissue in the first 0.5 h prior to the administration of sialyl Lewis X-oligosaccharide. It is consistent with the histopathological observation that the number of infiltrated polymorphonuclear leukocytes to lung in the delayed treatment − 0.5 h group was higher than that in the saline group (Fig. 3). Selectin inhibitors primarily block the adhesion of polymorphonuclear leukocytes to endothelial cells, and do not directly inhibit the subsequent acute inflammatory response such as destruction caused by the proteolytic enzymes and oxygen radicals released from the infiltrated polymorphonuclear leukocytes. Our observations are consistent with these mechanisms expected for selectin inhibitors. Similar effects were reported for the delayed treatment with nitric oxide (Bloomfield et al., 1997) and lisofylline, an inhibitor of the de novo generation of phosphatidic acid (Hasegawa et al., 1997). With the delayed administration protocols, these treatments also prevented further deterioration but did not lead to the full recovery to the control level as seen for sialyl Lewis X-oligosaccharide. The effects of these drugs including sialyl Lewis X-oligosaccharide were examined only for a period of several hours after lipopolysaccharide injection and their long-term pharmacological effects remain to be examined.

In conclusion, we demonstrated here for the first time the effect of delayed treatment with sialyl Lewis X-oligosaccharide against pulmonary dysfunction associated with lipopolysaccharide-induced acute lung injury in rabbits. Together with our previous report that demonstrated a prophylactic effect of the selectin inhibitor against lipopolysaccharide-induced acute lung injury in rabbits (Hayashi et al., 1999), the present study suggests that sialyl Lewis X-oligosaccharide may provide a potential therapeutic treatment for patients with acute lung injury, including acute respiratory distress syndrome associated with sepsis.

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