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The role of P-selectin, sialyl Lewis X and sulfatide in myocardial ischemia and reperfusion injury

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Abstract

The role of P-selectin and the ligands of selectins such as sialyl Lewis X and sulfatide was studied in a myocardial ischemia and reperfusion injury model. Anesthetized rabbits underwent the occlusion of coronary artery (30 min) followed by reperfusion (5 h). The inhibitory effect on myocardial ischemia and reperfusion injury was examined with infarct size normalized by area-at-risk. Intravenous administration of an anti-P-selectin monoclonal antibody, PB1.3 (2 mg/kg), reduced infarct size by 38%. Similarly, the administration of sialyl Lewis X-oligosaccharide (10 mg/kg) reduced infarct size by 53% significantly. Finally, the infarct size was significantly reduced by 39% in sulfatide-treated group (10 mg/kg). These results suggest that P-selectin plays an important role in myocardial ischemia and reperfusion injury and that the ligands of selectins, such as sialyl Lewis X-oligosaccharide and sulfatide, have cardioprotective effect on myocardial ischemia and reperfusion injury. © 1998 Elsevier Science B.V.

Keywords: Selectin; Anti-P-selectin antibody; Sialyl Lewis X; Sulfatide; Myocardial ischemia; Reperfusion injury

1. Introduction

Adhesion of polymorphonuclear leukocytes to endothelial cells is one of the most important steps involved in the development of myocardial ischemia and reperfusion injury (Forman et al., 1990). The adhesion of polymorphonuclear leukocytes consists of multiple steps facilitated by several species of adhesion molecules present both on polymorphonuclear leukocytes and endothelial cells (Granger and Kubes, 1994). In the inflammatory response, polymorphonuclear leukocytes initially interact with endothelial cells via selectins (E-, P-, L-selectin), which are responsible for tethering polymorphonuclear leukocytes (Tedder et al., 1995). Recently, the role of selectins in tethering was demonstrated in vivo in selectin deficient mice. After surgical stimulus, leukocytes rolling along endothelial cells was significantly impaired in P-selectin deficient and L-selectin deficient mice (Ley et al., 1995). After the tethering stage, polymorphonuclear leukocytes adhere firmly to endothelial cells by interactions between members of the integrin (CD11a/CD18, CD11b/CD18) and immunoglobulin superfamilies (intercellular adhesion molecule-1, -2; ICAM-1, -2), causing polymorphonuclear leukocytes to migrate into inflammatory sites (Granger and Kubes, 1994). Adhered or migrated polymorphonuclear leukocytes undergo activation and release several toxic substance including reactive oxygen species and proteolytic enzymes that subsequently cause tissue injury (Granger and Kubes, 1994; Lucchesi, 1990). Although roles of adhesion molecules in myocardial ischemia and reperfusion injury have been well-characterized using blocking monoclonal antibodies (mAbs) in feline (Ma et al., 1993; Simpson et al., 1988) and canine (Hartman et al., 1995; Weyrich et al., 1993), few studies have been carried out in rabbits.

The present study is focused on the role of selectins in myocardial necrosis in rabbits. Of three members of selectins, P-selectin, which is normally present in Weibel-Palade body of endothelial cells and α-granule of platelets, is rapidly translocated to the cell membrane upon stimulation such as thrombin, histamine or reactive oxygen species (Hattori et al., 1989; McEver, 1991; Patel et al., 1991). E-selectin is expressed on endothelial cells at several hours after stimulation by IL-1, or TNF-α (Bevilacqua et al., 1987, 1989). L-selectin is constitutively expressed on leukocytes (Jutila et al., 1989). All three selectins recog-

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nize a fucosylated, sialylated carbohydrate ligand, sialyl Lewis X (Foxall et al., 1992; Granger and Kubes, 1994). In vitro studies have revealed that sialyl Lewis X-oligosaccharide inhibits selectin-mediated polymorphonuclear leukocyte adhesion (Foxall et al., 1992; Phillips et al., 1990). In addition, recent studies have demonstrated that sulfatide, sulfated galactosylceramide, is recognized by all three selectins (Needham and Schnaar, 1993). These results suggest that sulfated galactosylceramide, a selectin ligand other than sialyl Lewis X, may also inhibit selectin-mediated inflammatory responses in vivo, although its effect on myocardial ischemia and reperfusion injury has not previously been reported.

The objectives of this study are: (1) to clarify the role of P-selectin in myocardial ischemia and reperfusion injury using anti-P-selectin mAb, and (2) to investigate the effect of sialyl Lewis X-oligosaccharide and sulfatide in the rabbit model.

2. Methods

2.1. Surgical preparation

Animal preparation was performed based on a minor modification of the previous report (Chiariello et al., 1988). Briefly, male New Zealand White rabbits (2.5–3.5 kg, Kitayama Labes, Ina, Japan) were anesthetized with sodium pentobarbital (40 mg/kg, i.v.). The rabbits were intubated through a tracheotomy and ventilated with a mechanical respirator (Animal respirator, Shinano, Tokyo, Japan). A catheter was inserted into right carotid artery to measure mean arterial blood pressure by a pressure transducer (P10EZ-1, Nihon Koden, Tokyo, Japan). Another catheter was inserted into the right jugular vein to maintain anesthesia by sustained infusion of sodium pentobarbital (1 mg/kg per h). The third catheter was placed in the right femoral vein for administration of drugs. A thoracotomy was performed through the fifth intercostal space and the heart was suspended in a pericardial cradle. A catheter was placed in the left atrium through the left atrial appendage. A 4-0 silk suture was tied around the large marginal branch of left circumflex coronary artery, approximately 0.5 cm from its origin. Electrocardiogram lead II was used to monitor heart rate and ST-segment elevation. The mean arterial blood pressure and electrocardiogram was continuously monitored by AP-641G and AC-601G units (Nihon Koden, Tokyo, Japan) during the experiment, respectively.

After surgical procedures, the rabbits were allowed to stabilize for 30 min. After baseline reading of mean arterial blood pressure and electrocardiogram recording, myocardial ischemia was induced by tightening the silk suture to occlude the vessel completely. Coronary artery occlusion was maintained for 30 min and ST-segment elevation was confirmed during the ischemia. Then occlusion was released and reperfusion was allowed for 5 h.

2.2. Experimental protocol

The rabbits were randomly divided into four major groups. (1) rabbits received saline (1 ml/kg) as vehicle, (2) anti-P-selectin mAb, PB1.3 (2 mg/kg or 5 mg/kg), (3) sialyl Lewis X-oligosaccharide (2 mg/kg, 10 mg/kg or 40 mg/kg), (4) sulfatide (10 mg kg). PB1.3 was administered by a single intravenous injection at 5 min prior to ischemia since the plasma half-life of the mAb has been demonstrated to be approximately 24 h in rabbits (Winn et al., 1993). For sialyl Lewis X-oligosaccharide or sulfatide, each drug was administered intravenously by two separate injections, a half volume at 5 min prior to ischemia and the remaining half at 5 min prior to reperfusion. Saline was administered by a single intravenous injection at 5 min prior to ischemia. Each drug did not affect ST-segment elevation during the ischemia.

2.3. Quantification of myocardial area-at-risk and necrotic area

At the end of reperfusion, the silk suture was tightened again and 1% Evans blue solution was infused quickly via catheter into left atrium. The heart was isolated and soaked in ice-cold saline. The left ventricle was dissected free from all other structures and cut into 13 slices that were parallel to the atrioventricular groove. The slices were observed as positive or negative staining areas and traced onto a transparent plastic sheets (Evans blue-negative staining area: area-at-risk). The slices, were then, incubated in a 1% solution of triphenyltetrazolium chloride for 10 min at 37°C and soaked in 10% formaldehyde neutral buffer solution overnight to clarify the staining. The triphenyltetrazolium chloride-positive or negative staining areas, were traced onto a transparent plastic sheet (triphenyltetrazolium chloride-negative staining area: necrotic area). The total left ventricle area, area-at-risk or necrotic area was measured by using an image analyzer (TIF-256R, Toyo Jozo, Kobe, Japan).

2.4. Number of circulating leukocytes

Blood samples (0.8 ml) were withdrawn from catheter and total leukocyte counts were measured by an automated blood cell counter, Sysmex F-800 (Toa Medical Electronics, Japan) at baseline, during the ischemic period and during reperfusion.

2.5. Drugs

PB1.3, anti-human P-selectin mAb (murine immunoglobulin G1) and sialyl Lewis X-oligosaccharide were kindly provided by Cytel (San Diego, USA). Cross-reactivity of PB1.3 to rabbit P-selectin is known by a demonstration that the mAb (2 mg/kg) impaired rabbit ear swelling induced by reperfusion injury and that expression of P-

Table 1
Summary of hemodynamic parameters

Experiment	Hemodynamic parameters	Treatment group	Baseline	Ischemia (30 min)	Hours post reperfusion					
					0.5 h	1 h	2 h	3 h	4 h	5 h
1	Mean arterial blood pressure (mmHg)	saline ($n=7$)	89±4	82±5	76±6	76±5	76±7	74±7	70±6	68±7
		PB1.3 2 mg/kg ($n=6$)	87±7	83±6	77±5	72±5	69±5	65±5	62±5	61±4
		PB1.3 5 mg/kg ($n=7$)	91±5	82±4	71±7	71±6	67±7	63±7	59±7	58±6
	Heart rate (beats/min)	saline ($n=7$)	284±8	274±11	277±7	266±5	245±6	238±5	231±6	225±9
		PB1.3 2 mg/kg ($n=6$)	281±12	276±8	264±8	255±9	243±10	228±14	221±16	209±17
		PB1.3 5 mg/kg ($n=7$)	275±9	272±10	260±10	256±12	247±11	232±9	224±9	219±10
2	Mean arterial blood pressure (mmHg)	saline ($n=8$)	102±2	98±2	92±2	92±2	84±3	76±3	69±4	65±4
		sialyl Lewis X 2 mg/kg ($n=6$)	100±2	85±3*	82±4	81±2	75±4	68±2	61±1	60±1
		sialyl Lewis X 10 mg/kg ($n=6$)	103±1	96±2	93±2	91±2	86±2	81±2	72±6	67±7
		sialyl Lewis X 40 mg/kg ($n=6$)	102±4	92±5	89±5	87±5	85±6	79±6	74±7	68±6
	Heart rate (beats/min)	saline ($n=8$)	297±10	290±9	284±9	280±9	268±8	257±8	247±8	237±8
		sialyl Lewis X 2 mg/kg ($n=6$)	308±9	286±8	293±6	292±9	285±8	280±10	275±11	264±8
		sialyl Lewis X 10 mg/kg ($n=6$)	282±10	271±8	275±11	270±8	265±7	261±7	258±6	243±6
		sialyl Lewis X 40 mg/kg ($n=6$)	294±7	284±11	269±12	267±10	261±11	259±11	250±8	244±9
3	Mean arterial blood pressure (mmHg)	saline ($n=8$)	101±2	97±1	92±2	92±2	84±3	76±3	69±4	64±4
		sialyl Lewis X 10 mg/kg ($n=6$)	103±1	96±2	93±2	91±2	86±2	81±2	72±6	67±6
		sulfatide 10 mg/kg ($n=7$)	99±4	90±3	84±4	82±5	75±6	68±5	59±4	58±4
	Heart rate (beats/min)	saline ($n=8$)	302±9	292±8	288±9	286±9	276±9	266±9	255±9	244±9
		sialyl Lewis X 10 mg/kg ($n=6$)	282±10	271±8	275±11	270±8	265±7	261±7	258±6	243±6
		sulfatides 10 mg/kg ($n=7$)	299±8	294±14	280±8	284±9	284±8	274±9	266±11	262±11

Each point represents mean ± S.E.
* $P < 0.05$ vs. saline-treated group by Dunnett's t-test.

Table 2
Peripheral leukocytes (cells/ul)

Experiment	Treatment group	Baseline	Ischemi (30 min)	Hours post reperfusion					
				0.5 h	1 h	2 h	3 h	4 h	5 h
1	saline ($n=7$)	2440±263	2160±193	2110±285	2010±234	2600±417	3190±325	3290±338	3360±424
	PB1.3 2 mg/kg ($n=6$)	2430±272	2200±103	2130±148	2180±221	2880±309	3170±198	3120±218	3200±301
	PB1.3 5 mg/kg ($n=7$)	3030±391	2100±273	1670±167	1770±167	2130±264	2610±370	2910±438	2740±399
2	saline ($n=8$)	3380±436	2630±308	2110±189	1990±200	2430±280	3010±320	3110±313	3060±305
	sialyl Lewis X 2 mg/kg ($n=6$)	3470±357	2780±326	2180±230	2350±269	2600±311	3020±425	3150±453	3150±339
	sialyl Lewis X 10 mg/kg ($n=6$)	3570±233	2720±149	2120±229	2070±329	2620±398	2930±439	3070±322	2980±441
	sialyl Lewis X 40 mg/kg ($n=6$)	3380±389	2300±186	1850±211	1980±303	2520±389	3270±351	3570±452	3800±543
3	saline ($n=8$)	3438±430	2650±302	2188±168	2038±181	2475±258	3125±288	3175±298	3088±300
	sialyl Lewis X 10 mg/kg ($n=6$)	3567±233	2717±149	2117±229	2067±329	2617±398	2933±439	3067±322	2980±441
	sulfatide 10 mg/kg ($n=7$)	3043±325	2300±223	1771±169	2029±263	2329±254	2900±367	3186±420	3600±504

Each point represents mean±S.E.
$*$ $P<0.05$ vs. saline-treated group by Dunnett's t-test.

selectin was detected immunohistochemically with the mAb (Winn et al., 1993). Sialyl Lewis X-oligosaccharide has previously used in various studies such as by Buerke et al. (1994), Lefer et al. (1994), Flynn et al. (1996), Han et al. (1995). Sulfatide (bovine brain) was obtained from Sigma Chemical (St. Louis, MO).

2.6. Statistical analysis

All values in the figures are presented as means ± standard errors. The data were subjected to analysis of variance (ANOVA) followed by Dunnett's *t*-test. Probabilities less than 0.05 were considered to be statistically significant.

3. Results

3.1. Effect of anti-P-selectin mAb

The effect of PB1.3 on hemodynamics change (mean arterial blood pressure or heart rate) was examined before ischemia, during ischemia period (30 min) and during reperfusion period (5 h). Mean arterial blood pressure and heart rate of all groups decreased in a equivalent degree throughout the experiment. PB1.3 caused no significant changes in any of the recorded parameters compared to the control group (Table 1), demonstrating that PB1.3 has no effect on hemodynamic parameters.

In order to determine whether PB1.3 has a leukopenic effect, which could also result in cardioprotection, we measured the number of circulating leukocytes at baseline, during ischemia and during reperfusion. The number of circulating leukocytes in the control group decreased at 0.5 h after reperfusion and gradually increased during the course of the experiment. PB1.3 had no significant effect on number of circulating leukocytes as compared to the control group (Table 2), indicating that PB1.3 did not induce leukopenia.

The effect of PB1.3 on infarct size is illustrated in Fig. 1. The size of areas subjected to ischemia (i.e., areas-at-risk) were similar between groups (saline-treated group, 43.1 ± 1.5%; PB1.3 (2 mg/kg)-treated group, 41.4 ± 1.8%; PB1.3 (5 mg/kg)-treated group, 42.4 ± 2.2%; $P > 0.05$), indicating that an equivalent degree of myocardial ischemia and reperfusion injury had occurred in each group. In contrast, infarct size, normalized by area-at-risk, was smaller in the PB1.3-treated group than the saline-treated group (saline-treated group, 42.8 ± 5.0%; PB1.3 (2 mg/kg)-treated group, 26.6 ± 9.0%; PB1.3 (5 mg/kg)-treated group, 30.8 ± 2.0%), although the reduction of infarct size was not achieved statistical significance. The reduction of infarct size was 38% (2 mg/kg) and 28% (5 mg/kg) by PB1.3, respectively. Similarly, infarct size, expressed as percentage of left ventricle area, was smaller in PB1.3-treated group than saline-treated group.

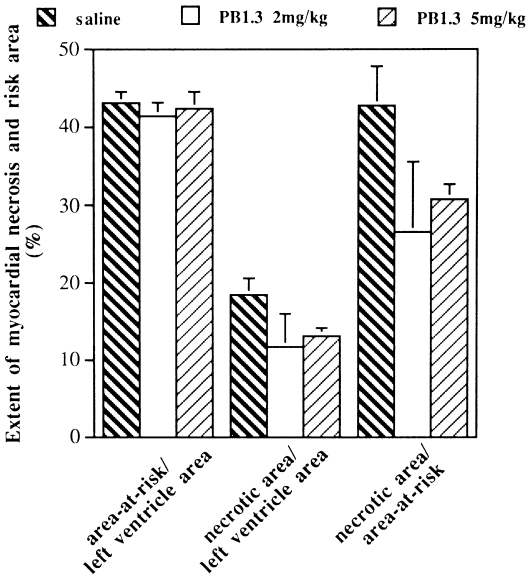

Fig. 1. Effect of anti-P-selectin mAb PB1.3 on myocardial infarction. The coronary artery was occluded for 30 min and reperfused for 5 h. After isolation of the heart, area-at-risk, left ventricle area and necrotic area were measured (see Section 2). Rabbits received 1 ml/kg of saline intravenously at 5 min prior to occlusion in saline-treated group ($n = 7$). Rabbits received 2 mg/kg ($n = 6$) or 5 mg/kg ($n = 7$) of PB1.3 intravenously at 5 min prior to occlusion in PB1.3-treated group.

3.2. Effect of sialyl Lewis X-oligosaccharide

The effect of sialyl Lewis X-oligosaccharide on hemodynamic changes (mean arterial blood pressure or heart rate) and circulating leukocytes was examined during the experimental period. Sialyl Lewis X-oligosaccharide had no significant effect on hemodynamic parameters or the number of circulating leukocytes as compared to the control group (Tables 1 and 2).

Fig. 2 shows the effects of sialyl Lewis X-oligosaccharide on infarct size. Area-at-risk was similar between groups (saline-treated group, 42.1 ± 2.6%; sialyl Lewis X-oligosaccharide (2 mg/kg)-treated group, 40.1 ± 3.8%; sialyl Lewis X-oligosaccharide (10 mg/kg)-treated group, 38.6 ± 1.8%; sialyl Lewis X-oligosaccharide (40 mg/kg)-treated group, 44.2 ± 1.5%; $P > 0.05$). Infarct size, normalized by area-at-risk, was significantly smaller in the sialyl Lewis X-oligosaccharide-treated groups than the saline-treated group (saline-treated group, 52.9 ± 5.0%; sialyl Lewis X-oligosaccharide (2 mg/kg)-treated group, 54.5 ± 6.1%; sialyl Lewis X-oligosaccharide (10 mg/kg)-treated group, 25.0 ± 5.4%; sialyl Lewis X-oligosaccharide (40 mg/kg)-treated group, 32.0 ± 3.9%). The reduction of infarct size was 53% (sialyl Lewis X-oligosaccharide 10 mg/kg; $P < 0.01$) and 40% (40 mg/kg; $P < 0.05$). Similarly, when expressed as a percentage of the left ventricle, infarct size was significantly smaller in the sialyl Lewis X-oligosaccharide (10 mg/kg)-treated group and was smaller in the sialyl Lewis X-oligosaccharide (40 mg/kg)-treated group than the saline-treated group.

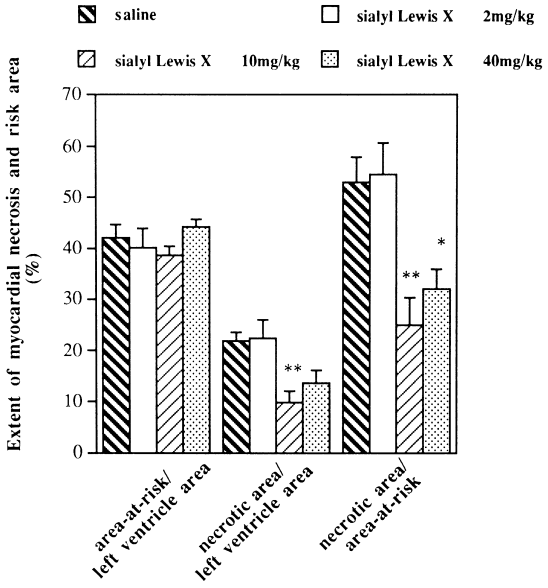

Fig. 2. Effect of sialyl Lewis X-oligosaccha-ride on myocardial infarction. The coronary artery was occluded for 30 min and reperfused for 5 h. After isolation of the heart, area-at-risk, left ventricle area and necrotic area were measured (see Section 2). Rabbits received 1 ml/kg of saline intravenously at 5 min prior to occlusion in saline-treated group ($n = 8$). Rabbits received 2 mg/kg ($n = 6$), 10 mg/kg ($n = 6$) or 40 mg/kg ($n = 6$) of sialyl Lewis X-oligosaccharide intravenously. Half volume of sialyl Lewis X-oligosaccharide was administered at 5 min prior to occlusion and remaining half was administered at 5 min prior to reperfusion. Statistical significance: $* P < 0.05$, $** P < 0.01$ vs. saline-treated group by Dunnett's *t*-test.

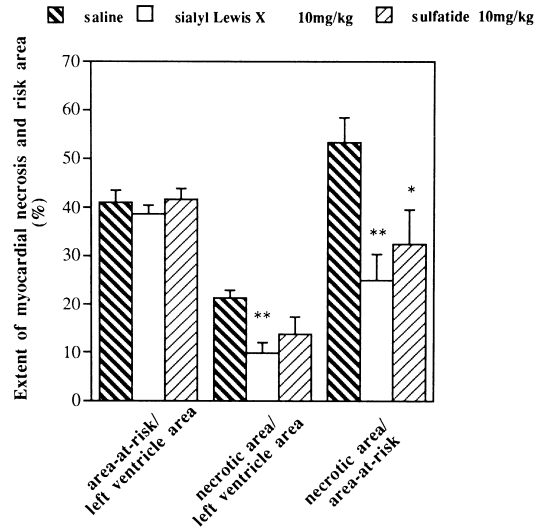

Fig. 3. Effect of sialyl Lewis X-oligosaccha-ride and sulfatide on myocardial infarction. The coronary artery was occluded for 30 min and reperfused for 5 h. After isolation of the heart, area-at-risk, left ventricle area and necrotic area were measured (see Section 2). Rabbits received 1 ml/kg of saline intravenously at 5 min prior to occlusion in saline-treated group ($n = 8$). Rabbits received 10 mg/kg of sialyl Lewis X-oligosaccharide ($n = 6$) or sulfatide ($n = 7$) intravenously. Half volume of sialyl Lewis X-oligosaccharide or sulfatide was administered at 5 min prior to occlusion and remaining half was administered at 5 min prior to reperfusion. Statistical significance: $* P < 0.05$, $** P < 0.01$ vs. saline-treated group by Dunnett's *t*-test.

3.3. Effect of sulfatide

As demonstrated for PB1.3 and sialyl Lewis X-oligosaccharide, sulfatide also had no significant effect on hemodynamic parameters (Table 1) or the number of circulating leukocytes (Table 2). The effects of sialyl Lewis X-oligosaccharide or sulfatide on infarct size is shown in Fig. 3. Both inhibitors were administered at the same dose of 10 mg/kg. Area-at-risk appeared to be the equivalent degree between groups (saline-treated group, $41.0 \pm 2.5\%$; sialyl Lewis X-oligosaccharide-treated group, $38.6 \pm 1.8\%$; sulfatide-treated group, $41.7 \pm 2.2\%$; $P > 0.05$). When normalized by area-at-risk, infarct size was significantly less in the sialyl Lewis X-oligosaccharide and the sulfatide-treated groups than the saline-treated group (saline-treated group, $53.3 \pm 5.1\%$; sialyl Lewis X-oligosaccharide-treated group, $25.0 \pm 5.4\%$; sulfatide-treated group, $32.4 \pm 7.1\%$). The reduction of infarct size was 53% for sialyl Lewis X-oligosaccharide ($P < 0.01$) and 39% for sulfatide ($P < 0.05$), respectively. Similarly, infarct size was significantly smaller in sialyl Lewis X-oligosaccharide-treated group and was smaller in sulfatide-treated group than saline-treated group, when the infarct size was expressed as percentage of left ventricle area.

4. Discussion

Polymorphonuclear leukocytes have been implicated in pathogenesis of myocardial ischemia and reperfusion injury (Forman et al., 1990). More direct evidence was provided by the demonstration that the depletion of polymorphonuclear leukocytes ameliorates the acute myocardial infarction in a canine model (Litt et al., 1989; Romson et al., 1983). Recently, intensive studies have demonstrated that families of adhesion molecules such as selectin, integrin, immunoglobulin superfamilies, are involved in the multiple steps of the interaction between polymorphonuclear leukocytes and endothelial cells, leading to the migration of polymorphonuclear leukocytes into inflammatory sites (Granger and Kubes, 1994). In myocardial ischemia and reperfusion injury, studies using mAbs against adhesion molecules that are present on polymorphonuclear leukocytes and endothelial cells demonstrated that anti-adhesion therapy was beneficial at least at the experimental level. In a feline model, anti-L-selectin mAb (Ma et al., 1993) or anti-P-selectin mAb (Weyrich et al., 1993) attenuated myocardial necrosis. In a canine model, anti-CD11b/CD18 mAb (Simpson et al., 1988) or anti-ICAM-1 mAb (Hartman et al., 1995) reduced infarct size. In the present study, we examined myocardial ischemia and reperfusion injury in a rabbit model. While many studies have been conducted in feline or canine models, few studies have been carried out in a rabbit model to date.

We demonstrated that an anti-P-selectin mAb PB1.3 reduced infarct size by approximately 38% (2 mg/kg) and

28% (5 mg/kg) in rabbits undergoing 30 min of ischemia followed by 5 h reperfusion (Fig. 1). PB1.3 did not affect hemodynamic parameters or the number of peripheral leukocytes (Tables 1 and 2), suggesting that the cardioprotective effect observed with PB1.3 was not resulted from leukopenia. PB1.3 inhibits adhesion of polymorphonuclear leukocytes to coronary artery endothelium stimulated with thrombin or histamine, which induces expression of P-selectin on endothelial cells (Weyrich et al., 1993). Therefore, the cardioprotective effect of PB1.3 in our study could be explained by the similar mechanism. The involvement of P-selectin in myocardial ischemia and reperfusion injury has previously been investigated in a feline model in which P-selectin is maximally expressed 20 min after reperfusion in coronary venules as detected immunohistochemically (Weyrich et al., 1995). In this model, PB1.3 reduced infarct size by approximately 57% (Weyrich et al., 1993). Also, PB1.3 preserved coronary flow and preserved myocardial contractile function in a canine myocardial ischemia and reperfusion model (Chen et al., 1994). In addition, we have recently demonstrated that an anti-rat P-selectin mAb, ARP2-4, attenuated infarct size in a rat myocardial ischemia and reperfusion injury model (Tojo et al., 1996). The results in the present work in rabbits are consistent with these previous work and demonstrate the importance of P-selectin in the pathogenesis of acute postischemic myocardial injury.

P- and E-selectins on the endothelial cells, and L-selectin on polymorphonuclear leukocytes interact with the carbohydrate ligand, sialyl Lewis X, which inhibits polymorphonuclear leukocyte adhesion in vitro (Foxall et al., 1992). In addition, sulfatide (3-sulfated galactosylceramide) was recently shown to be a selectin ligand and to bind to all three selectins (Needham and Schnaar, 1993). Although a number of studies have reported that sulfatide inhibits polymorphonuclear leukocyte adhesion mediated by selectins in vitro (Needham and Schnaar, 1993; Suzuki et al., 1993; Todderud et al., 1992), few studies have demonstrated the anti-inflammatory effect in vivo (Mulligan et al., 1995; Ohnishi et al., 1996). In this report, we demonstrated the effect of two soluble selectin ligands, sialyl Lewis X-oligosaccharide and sulfatide, on myocardial ischemia and reperfusion injury. Sialyl Lewis X-oligosaccharide significantly reduced the infarct size by 53% (10 mg/kg) and 40% (40 mg/kg) in a rabbit myocardial ischemia and reperfusion model at 5 h of reperfusion (Fig. 2). Moreover, we demonstrated that the same dose of sulfatide (10 mg/kg) significantly reduced infarct size by 39% (Fig. 3). Neither sialyl Lewis X-oligosaccharide nor sulfatide affected hemodynamic parameters (Table 1). Also, the inhibitory effect on infarct size cannot be attributed to changes in circulating leukocyte numbers (Table 2), suggesting that myocardial salvage was achieved by the protective effect of sialyl Lewis X-oligosaccharide or sulfatide. Previous studies in feline and canine models have demonstrated the effect of soluble sialyl Lewis X on myocardial ischemia and reperfusion injury. Sialyl Lewis X-oligosaccharide reduced infarct size by 83%, inhibited polymorphonuclear leukocyte adhesion to endothelial cells in the feline model (Buerke et al., 1994) and reduced infarct size by 67%, ameliorated polymorphonuclear leukocyte accumulation in myocardium by 63% in the canine model (Lefer et al., 1994). In contrast, recent study in a canine model has revealed that sialyl Lewis X-oligosaccharide does not reduce infarct size in prolonged reperfusion period (48 h) (Gill et al., 1996). The ineffectiveness of sialyl Lewis X-oligosaccharide in their report may be due to a single bolus administration of the drug, resulting in insufficient blood concentration of sialyl Lewis X-oligosaccharide to block selectin-mediated polymorphonuclear leukocyte adhesion in the condition of the prolonged reperfusion period. In fact, the administration of sialyl Lewis X-oligosaccharide by a bolus injection followed by the continuous infusion for 24 h has reduced infarct size and polymorphonuclear leukocyte accumulation in canine model in 48 h reperfusion (Flynn et al., 1996). The efficacy of sialyl Lewis X-oligosaccharide in a rabbit model has been demonstrated. Sialyl Lewis X-oligosaccharide (25 mg/kg bolus i.v. followed by 50 mg/kg infusion over 10 h) reduced rabbit ear swelling and necrosis, which was induced by ischemia and reperfusion (Han et al., 1995). Our observations are consistent with these previous reports and clearly demonstrated that sialyl Lewis X-oligosaccharide has cardioprotective properties in myocardial ischemia and reperfusion injury in rabbits.

Although we did not examine the accumulation of polymorphonuclear leukocyte in the myocardium at risk and in the necrosis area in the present study, protective effect of the selectin inhibitors observed here is likely resulted from the inhibition of polymorphonuclear leukocyte accumulation in the rabbit heart. It has previously been demonstrated in various studies that the protective effect of selectin inhibitors were associated with the reduced level of polymorphonuclear leukocyte accumulation in the injured tissues. For example, in ischemia and reperfusion injury models, polymorphonuclear leukocyte accumulation was reduced in the canine heart by PB1.3 (Chen et al., 1994) and sialyl Lewis X-oligosaccharide (Lefer et al., 1994). Similar results were also obtained in ischemia and reperfusion injury in kidney (Davenpeck et al., 1994) and liver (Misawa et al., 1996). In addition, sulfatide was shown to reduce polymorphonuclear leukocyte accumulation in acute lung injury models in rats (Mulligan et al., 1995) as previously demonstrated with PB1.3 (Mulligan et al., 1992) and sialyl Lewis X-oligosaccharide (Mulligan et al., 1993). Finally, we have recently demonstrated using an anti-rat P-selectin mAb, ARP2-4, that reduction of footpad swelling was associated with the inhibition of polymorphonuclear leukocyte accumulation in a rat dermal injury induced by the Arthus reaction (Ohnishi et al., 1996).

In addition to P-selectin, L-selectin has been shown to be involved in myocardial ischemia and reperfusion injury.

An anti-L-selectin mAb, DREG-200, attenuated myocardial necrosis, coronary endothelial dysfunction and polymorphonuclear leukocyte accumulation in myocardium in a feline model (Ma et al., 1993). The pathophysiological role of E-selectin in myocardial ischemia and reperfusion injury, however, remains to be clarified. A mAb directed against E-selectin showed no cardioprotection in a primate model (Winquist et al., 1992), whereas an anti-E-selectin mAb, BBIG-E5, reduced infarct size and serum creatine phosphokinase activity as well as polymorphonuclear leukocyte accumulation in a rodent model (Altavilla et al., 1994). Since L-selectin is constitutively expressed on polymorphonuclear leukocytes (Jutila et al., 1989), the effect of sialyl Lewis X-oligosaccharide and sulfatide could be at least in part attributed to blockade of L-selectin-mediated polymorphonuclear leukocyte adhesion. Therefore, the cardioprotective effect of sialyl Lewis X-oligosaccharide and sulfatide seen in this study could be facilitated mainly by blockade of adhesion via P- and L-selectins, but inhibition of E-selectin-mediated adhesion cannot be eliminated. Sialyl Lewis X-oligosaccharide and sulfatide, which block all selectins, could result in greater myocardial salvage as compared to mAbs that inhibits only a specific selectin and therefore, this could possibly explain the larger cardioprotective effect obtained by sialyl Lewis X-oligosaccharide (10 mg/kg; 53%) and sulfatide (10 mg/kg; 39%) than that observed by a mAb, PB1.3 (2 mg/kg; 38%).

To our knowledge, this is the first demonstration of the protective effect of sulfatide on myocardial ischemia and reperfusion injury in animal model. Protective effects of sulfatide have been shown in rat lung injury models, induced by cobra venom factor and immunoglobulin G immune complex (Mulligan et al., 1995) as well as dermal injury induced by the Arthus reaction (Ohnishi et al., 1996). The involvement of selectins in these models has previously been demonstrated using anti-selectin mAbs (Mulligan et al., 1991; Mulligan et al., 1992; Ohnishi et al., 1996). Our present study clearly demonstrated the protective effect of sulfatide on myocardial ischemia and reperfusion injury.

In summary, we have demonstrated the cardioprotective effects of anti-P-selectin mAb, sialyl Lewis X-oligosaccharide and sulfatide in rabbits and that myocardial ischemia and reperfusion injury is mediated by selectins, particularly by P-selectin.

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