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Inhibition of P-selectin attenuates neutrophil-mediated myocardial dysfunction in isolated rat heart

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Abstract

The expression of P-selectin on postischemic endothelium after reperfusion has been shown to trigger neutrophil attachment and the subsequent inflammatory responses. Extensive studies have demonstrated that P-selectin is involved in the progression of neutrophil-mediated myocardial infarction and no-reflow phenomenon. In the present study, we examined the effects of selectin inhibitors, sialyl Lewis X-oligosaccharide and anti-P-selectin monoclonal antibody, PB1.3 on neutrophil-dependent left ventricular dysfunction in isolated rat heart. The hearts were subjected to global ischemia for 20 min and then reperfused for 45 min with rat plasma in the presence of human neutrophils during the first 5 min of the reperfusion. Left ventricular developed pressure and other parameters of the left ventricular function deteriorated throughout the reperfusion period in a neutrophil-dependent manner. In contrast, the coronary flow was reduced early on (<15 min) but recovered to the level in the hearts reperfused with no neutrophils 45 min after the reperfusion. We examined the effects of selectin inhibitors under experimental conditions in which the hearts were perfused with 30 million neutrophils. The treatment with sialyl Lewis X-oligosaccharide at a dose of 0.3 mg/min resulted in amelioration of left ventricular developed pressure to $57.2 \pm 14\%$, compared to $26.1 \pm 4.3\%$ in the saline-treated group ($P < 0.05$). Similarly, the treatment with mouse anti-human P-selectin monoclonal antibody (IgG1) PB1.3 at a dose of 0.6 mg/min resulted in the prominent recovery of left ventricular developed pressure after 45 min of reperfusion ($59.9 \pm 9.3\%$ vs. $26.1 \pm 4.3\%$ in the saline-treated group, $P < 0.05$). PB1.3 also attenuated the elevation of left ventricular end-diastolic pressure compared to that of the saline-treated group during the reperfusion period. Moreover, the treatment with PB1.3 ameliorated the recovery of coronary flow until 10 min after the reperfusion and the recovery of coronary flow 10 min after the reperfusion was $55.2 \pm 9.2\%$, as compared to $28.2 \pm 7.7\%$ in saline-treated hearts ($P < 0.05$). To our knowledge, this is the first direct demonstration that the specific inhibition of P-selectin results in the inhibition of neutrophil-mediated left ventricular dysfunction or myocardial stunning.

Keywords: P-selectin; Ischemia and reperfusion injury; Neutrophil; Stunning

1. Introduction

Selectins are a family of cell adhesion molecules responsible for adhesive interactions of leukocytes and platelets with endothelial cells. Three known selectins, E-, P- and L-selectins are structurally related but differ in their pattern of expression (Bevilacqua and Nelson, 1993; Tedder et al., 1995). For example, the activation of endothelial cells by histamine and thrombin results in the translocation of P-selectin from Weibel–Palade bodies to the cell surface within 10–20 min. The expression of E-selectin in endothelial cells occurs via de novo synthesis 4 h after stimulation of the cells by cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1. In contrast, L-selectin is expressed constitutively on leukocytes. All selectins bind to ligands containing sialylated, fucosylated carbohydrate moieties such as sialyl Lewis X (Tedder et al., 1995; Varki, 1997).

Previous studies have demonstrated that the specific adhesion between neutrophils and the endothelial cells via

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selectins is involved in the pathogenesis of various inflammatory disorders, transplant rejection, ischemia and reperfusion injury and thrombosis (Henricks and Nijkamp, 1998). In addition, patients with leukocyte adhesion deficiency syndrome type 2 have genetic defects in the fucose metabolism resulting in failure to synthesize selectin ligands and consequently, display impaired neutrophil emigration to inflammatory tissues, which is caused by the inability of neutrophils to bind to E- and P-selectins (Phillips et al., 1995). The role of selectins has been studied particularly in the area of myocardial ischemia-reperfusion injury. It has been reported that anti-P-selectin monoclonal antibody, PB1.3, and a ligand for selectins, sialyl Lewis X-oligosaccharide, significantly reduced myocardial necrosis after ischemia-reperfusion in feline (Weyrich et al., 1993; Buerke et al., 1994) and canine models (Lefer et al., 1994, 1996). Similarly, anti-L-selectin antibody was protective in a feline model (Ma et al., 1993). In addition, Seko et al. (1996) reported that administration of a synthetic oligopeptide containing the common sequence among E-, P- and L-selectins significantly reduced the area of myocardial infarction in rat. Moreover, we have demonstrated that treatment with anti-rat P-selectin monoclonal antibody resulted in reduction of myocardial necrosis in rat (Tojo et al., 1996). Taken together, these reports have demonstrated the roles of selectins in the pathogenesis of myocardial necrosis after ischemia-reperfusion.

In addition to myocardial necrosis, myocardial dysfunction followed by ischemia-reperfusion are speculated to be caused by the activation of neutrophils. First, a significant proportion of the capillaries fails to reperfuse upon reinstitution of the blood flow despite the restoration of adequate perfusion pressure, a phenomenon termed 'no-reflow'. No-reflow was prevented by neutrophil depletion in the postischemic heart (Engler et al., 1983), brain (Carden et al., 1990) and skeletal muscles (Del Zoppo et al., 1991). Jerome et al. (1994) demonstrated that the inhibition of P-selectin improved the no-reflow phenomenon in canine skeletal muscles using monoclonal antibody PB1.3. Second, postischemic ventricular dysfunction or myocardial stunning is a mechanical dysfunction that persists after reperfusion despite the absence of irreversible organic damage (Bolli, 1990; Williams, 1996). In a canine model, depletion of neutrophils prevented postischemic contractile dysfunction (Engler and Covell, 1987; Westlin and Mullane, 1989). In contrast to the necrosis and no-reflow phenomenon, however, the roles of selectins in myocardial stunning have not been fully elucidated.

In the present study, we employed a left ventricular dysfunction model using isolated rat hearts, which were subjected to global ischemia for 20 min followed by reperfusion with rat plasma as well as human neutrophils. We then examined whether the blockade of selectins reduced the neutrophil-mediated left ventricular dysfunction using sialyl Lewis X-oligosaccharide as well as the anti-P-selectin monoclonal antibody.

2. Materials and methods

2.1. Isolated heart perfusion

All procedures related to the use of animals in the present study were reviewed and approved by the Institutional Animal Care and Use Committee at Sumitomo Pharmaceuticals Research Center (Osaka, Japan). We have used an isolated rat heart model with a modification of a previously described method (Shandelya et al., 1993). Male Wister–ST rats weighing 300 to 400 g were obtained from Japan SLC (Hamamatsu, Japan). Rats were given heparin (700 units/kg) and pentobarbital (50 mg/kg) intraperitoneally. Hearts were removed rapidly and perfused by the Langendorff method at a constant pressure of 80 mm Hg with modified Krebs–Henseleit buffer (in mM): NaCl 119; KCl 4.59; $NaHCO_3$ 24.9; $MgSO_4$ 1.2; $CaCl_2$ 1.31; KH_2PO_4 1.21, and glucose 11. The perfusate was filtered through a cellulose nitrate membrane (0.65 μm pore size) (Nihon Millipore, Yonezawa, Japan) and bubbled with 95% O_2/5% CO_2. The temperature of the whole system was maintained at 37 ± 2°C using a water bath. Two side arms of the perfusion line located just proximal to the aortic cannula allowed infusion of human neutrophils and rat plasma directly into the coronary vessels. An ultrasonic flow probe (2N Transonic Systems Ithaca, NY) was attached in the middle of the perfusion line to measure coronary flow. To assess the left ventricular function, a latex balloon was inserted into the left ventricular cavity through an incision in the left atrium. The balloon was connected to a pressure transducer (DX-360, Nihon Koden, Tokyo, Japan) and the signal was amplified, converted digitally and displayed on a computer screen using MacLAB™ system (ADInstruments, NSW, Australia). The balloon was initially inflated to produce left ventricular end-diastolic pressure of 10 mm Hg.

2.2. Preparation of human neutrophil and rat plasma

Approximately 25 ml of heparinized blood was drawn from healthy volunteer donors and overlaid on 15 ml of Mono-poly-resolving medium (Dainippon Pharma Osaka, Japan). The tubes were centrifuged at room temperature for 30 min at $1500 \times g$. The neutrophil-rich layer was collected, washed with phosphate buffered saline twice, resuspended in 3 ml of distilled water and left for 20 s to lyse red blood cells. Then neutrophils were resuspended in Hank's buffer containing 10 mM of Hepes (in mM): NaCl 136.8; KCl 5.36; $NaHCO_3$ 4.16; $MgCl_2$ 0.49; $MgSO_4$ 0.40; $CaCl_2$ 1.26; KH_2PO_4 0.44; Na_2PO_4 0.33, and glucose 5.55. The cells were counted and kept on ice until the subsequent experiments. This procedure yielded neu-

trophils with a purity of $>95\%$ and the remaining cell population appeared to be monocytic cells with no contamination of platelets as determined by the microscopic cell typing after Giemsa staining. Rat blood was collected from the abdominal aorta with a heparinized syringe and immediately centrifuged for 10 min at $1500 \times g$. The supernatant was collected and used as rat plasma.

2.3. Experimental protocol

The preischemic control values were measured for left ventricular developed pressure, left ventricular end-diastolic pressure and coronary flow after an equilibration period of 15–20 min. The hearts were subjected to global ischemia for 20 min. When the perfusion was reinitiated, human neutrophils and rat plasma were also infused individually through two sidearms for the first 5 min. The perfusion was continued with the buffer alone for a total of 45 min. The postischemic cardiac functions and coronary flow were measured at 5, 10, 15, 20, 25, 30 and 45 min after the reperfusion. For the control group, plasma alone was added at the onset of reperfusion. In order to minimize the variability of the degree of myocardial failure in the preparation caused by the various experimental conditions such as the source of neutrophils from different donors, experiment of each compared group was performed on the same day using neutrophils from the same donor.

Selectin inhibitors, a murine anti-human P-selectin monoclonal antibody (IgG1) PB1.3 or a sialyl Lewis X-oligosaccharide were added to the rat plasma and infused during the first 5 min of reperfusion at doses of 0.6 and 0.3 mg/min, respectively. These selectin inhibitors have previously shown protective effects in various species such as in feline (Weyrich et al., 1993; Buerke et al., 1994), rabbit (Winn et al., 1993; Yamada et al., 1998) and canine models (Lefer et al., 1994, 1996; Ueyama et al., 1997). Moreover, the protective effects of PB1.3 and the sialyl Lewis X-oligosaccharide in lung injury models were shown in rat (Mulligan et al., 1992, 1993a,b). We have recently demonstrated that PB1.3 reduced the edema formation in rat dermal injury induced by the Arthus reaction (Ohnishi et al., 1996), and that PB1.3 and sialyl Lewis X-oligosaccharide attenuated myocardial necrosis in rabbit (Yamada et al., 1998). Thus, the two selectin inhibitors used in the present study are known to cross-react with the selectin molecules in respective animal species. PB1.3 and sialyl Lewis X-oligosaccharide were obtained from Cytel (San Diego, CA, USA).

2.4. Statistical analysis

Data were expressed as the mean $\pm$ S.E.M. Unpaired Student's *t*-test was used to compare the differences between the percent recovery of the baseline values between two groups. Statistical significance was accepted at the 0.05 level.

3. Results

3.1. Neutrophil-mediated left ventricular dysfunction

We have developed an isolated rat heart model perfused with human neutrophils. The isolated hearts were subjected to global ischemia for 20 min and then reperfused for 45 min with rat plasma in the presence or absence of human neutrophils during the first 5 min of the reperfusion. To confirm that the myocardial dysfunction is caused by an addition of neutrophils, we first examined whether the extent of dysfunction could be altered by the number of neutrophils added in our model. The results are summarized in Table 1. In hearts perfused with neutrophils, a marked decrease in the recovery of left ventricular developed pressure was observed at 45 min after the reperfusion. The final recovery of left ventricular developed pressure after 45 min of reperfusion in hearts of the control group was 86.0% as compared with 43.1% in hearts treated with 30 million neutrophils ($P < 0.01$) and 46.9% with 45 million neutrophils ($P < 0.05$), respectively (Table 1). There was no difference between the groups given 30 million and 45 million cells, suggesting that the neutrophil-mediated injury plateaued at a dose of 30 million cells. Similar results were obtained for left ventricular end-diastolic pressure. The neutrophil-perfused hearts showed significantly higher left ventricular end-diastolic pressure (Table 1). The final value of left ventricular

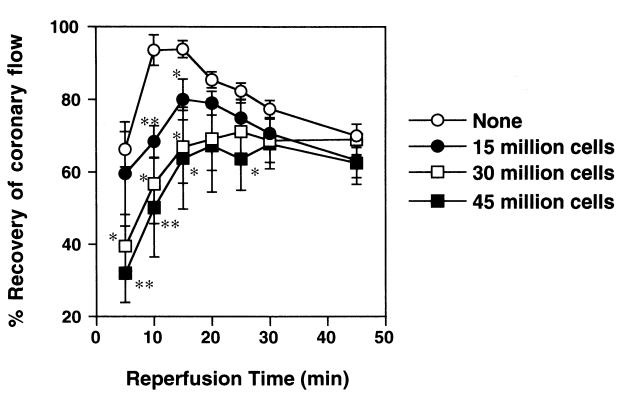

Fig. 1. The effects of various numbers of neutrophils on coronary flow. Hearts were reperfused with rat plasma with no human neutrophils (open circles); with 15 million human neutrophils (closed circles); with 30 million human neutrophils (open squares); or with 45 million human neutrophils (closed squares). Statistical significance: $*\ P < 0.05$, $**\ P < 0.01$ vs. plasma alone group.

Table 1
The effects of various numbers of neutrophils on physiological parameters 45 min after the reperfusion

	LVDP (% recovery)	LVEDP (mm Hg)	CF (% recovery)	dp/dt max (% recovery)	dp/dt min (% recovery)	HR (% recovery)	PRP (% recovery)
Plasma alone ($n=7$)	86.0 ± 12	13.1 ± 8.0	70.0 ± 3.2	86.0 ± 12	82.9 ± 13	90.5 ± 4.0	77.7 ± 11
Plasma plus 15 million PMNs ($n=5$)	70.5 ± 7.5	33.2 ± 9.5	63.2 ± 4.8	67.7 ± 6.3	66.1 ± 6.0	71.8 ± 10	49.0 ± 6.3
Plasma plus 30 million PMNs ($n=4$)	43.1 ± 8.6 ($P<0.01$)	50.6 ± 7.6 ($P<0.01$)	69.0 ± 4.2	35.7 ± 9.3 ($P<0.05$)	45.8 ± 9.9	85.7 ± 4.3	37.7 ± 8.7 ($P<0.05$)
Plasma plus 45 million PMNs ($n=6$)	46.9 ± 6.5 ($P<0.05$)	49.0 ± 5.8 ($P<0.01$)	62.5 ± 5.8	43.4 ± 7.1 ($P<0.05$)	46.7 ± 7.2	86.9 ± 14	42.0 ± 11

Hearts were reperfused with or without various numbers of human neutrophils and the indicated parameters examined.
Values are the mean ± S.E.M.
LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; CF, coronary flow; dp/dt max, positive peak of the first derivative of LVDP; dp/dt min, negative peak of the first derivative of LVDP; HR, heart rate; PRP, pressure-rate product.
P-value vs. plasma alone group.

Table 2
The effects of sialyl Lewis X-oligosaccharide on physiological parameters 45 min after the reperfusion

	LVDP (% recovery)	LVEDP (mm Hg)	CF (% recovery)	dp/dt max (% recovery)	dp/dt min (% recovery)	HR (% recovery)	PRP (% recovery)
Saline ($n=9$)	26.1 ± 4.3	68.2 ± 4.8	47.7 ± 6.0	25.7 ± 4.3	26.2 ± 4.9	76.9 ± 8.5	20.4 ± 4.0
Sialyl Lewis X-oligosaccharide ($n=6$)	57.2 ± 14 ($P<0.05$)	41.8 ± 9.7 ($P<0.05$)	58.1 ± 8.5	54.3 ± 14 ($P<0.05$)	51.7 ± 15 ($P=0.08$)	76.0 ± 11	47.2 ± 15

Hearts were reperfused with 30 million human neutrophils with or without sialyl Lewis X-oligosaccharide at a dose of 0.3 mg/ml and the indicated parameters examined.
Values are the mean ± S.E.M.
LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; CF, coronary flow; dp/dt Max, positive peak of the first derivative of LVDP; dp/dt Min, negative peak of the first derivative of LVDP; HR, heart rate; PRP, pressure-rate product.
P-value vs. saline-treated group.

end-diastolic pressure at the end of the reperfusion period in the control group was 13.1 ± 8.0 mm Hg as compared to 50.6 ± 7.6 mm Hg in hearts treated with 30 million neutrophils ($P < 0.01$) and 49.0 ± 5.8 mm Hg in those given 45 million neutrophils ($P < 0.01$), respectively. The deterioration of the positive peak of the first derivative of left ventricular developed pressure (dp/dt max) caused by neutrophils appeared to be of similar magnitude to that observed for left ventricular developed pressure (Table 1). In contrast, coronary flow in neutrophil-perfused groups recovered to the level of the control after 45 min (Table 1). Although the left ventricular functions such as developed pressure and end-diastolic pressure were deteriorated by neutrophils throughout the reperfusion period, coronary flow was attenuated by neutrophils earlier (< 15 min) after the reperfusion but had recovered by 45 min (Fig. 1). Recovery of coronary flow 10 min after reperfusion in the control group was $93.5 \pm 4.1\%$ as compared to $68.4 \pm 4.3\%$ in hearts treated with 15 million neutrophils ($P < 0.01$), $56.7 \pm 11\%$ in those treated with 30 million neutrophils ($P < 0.05$) and $50.1\% \pm 13$ in those perfused with 45 million neutrophils ($P < 0.01$), respectively (Fig. 1).

3.2. Effect of sialyl Lewis X-oligosaccharide on cardiac function

To examine the effects of selectin inhibitors, we chose an experimental condition in which the hearts were perfused with 30 million neutrophils. First, we examined the effect of sialyl Lewis X-oligosaccharide on this model. Sialyl Lewis X-oligosaccharide at a dose of 0.3 mg/min was infused during the first 5 min of reperfusion with neutrophils. The preischemic baseline values of left ventricular function such as developed pressure and coronary flow appeared to be equivalent between the two groups studied (data not shown). At the end of the reperfusion period, the treatment with sialyl Lewis X-oligosaccharide ameliorated the recovery of left ventricular developed pressure to $57.2 \pm 14\%$, compared to $26.1 \pm 4.3\%$ in the saline-treated group ($P < 0.05$) (Table 2). Similarly, sialyl Lewis X-oligosaccharide showed protective effects on other parameters including left ventricular end-diastolic pressure, dp/dt max and pressure-rate product (Table 2).

3.3. Effect of anti-P-selectin monoclonal antibody on cardiac function

Because sialyl Lewis X-oligosaccharide blocks cell adhesion mediated by E-, P- and L-selectins, we evaluated the effect of anti-P-selectin monoclonal antibody, PB1.3 to examine the role of P-selectin in this model. PB1.3 was reported not to cross-react with E- and L-selectins and its

Table 3
The effects of anti-P-selectin monoclonal antibody (PB1.3) on physiological parameters 45 min after the reperfusion

	LVDP (% recovery)	LVEDP (mm Hg)	CF (% recovery)	dp/dt Max (% recovery)	dp/dt Min (% recovery)	HR (% recovery)	PRP (% recovery)
Saline ($n = 6$)	30.9 ± 6.0	64.0 ± 5.7	66.5 ± 11	24.0 ± 4.6	36.0 ± 7.7	94.7 ± 2.1	28.8 ± 5.3
Anti-P-selectin antibody (PB1.3) ($n = 6$)	59.9 ± 9.3 ($P < 0.05$)	41.8 ± 6.9 ($P < 0.05$)	64.0 ± 5.9	60.4 ± 9.5 ($P < 0.01$)	59.1 ± 8.7 ($P = 0.07$)	92.9 ± 2.8	55.6 ± 8.6 ($P < 0.05$)

Hearts were reperfused with 30 million human neutrophils with or without PB1.3 at a dose of 0.6 mg/ml and the indicated parameters examined.
Values are the mean ± S.E.M.
LVDP, left ventricular developed pressure; LVEDP, left ventricular end-diastolic pressure; CF, coronary flow; dp/dt Max, positive peak of the first derivative of LVDP; dp/dt Min, negative peak of the first derivative of LVDP; HR, heart rate; PRP, pressure-rate product.
P-value vs. saline-treated group.

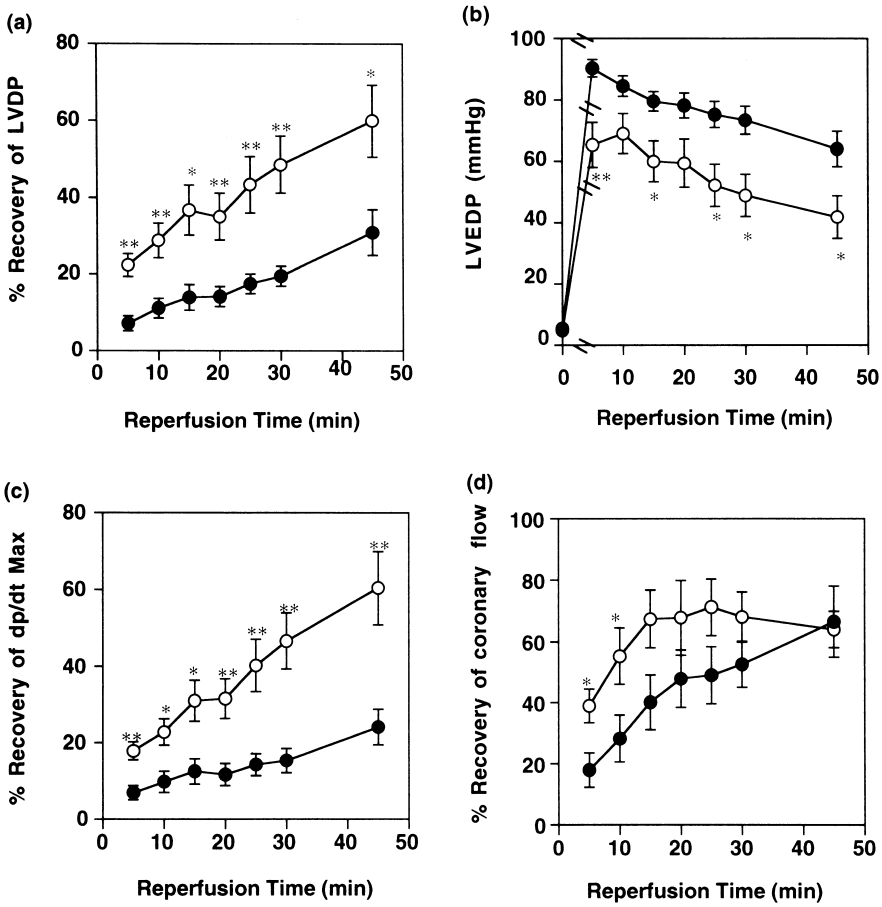

Fig. 2. The effects of PB1.3 on the contractile dysfunction and coronary flow. Hearts were reperfused with 30 million human neutrophils with (open circles) or without (closed circles) PB1.3 at a dose of 0.6 mg/ml. The effects of PB1.3 on the recovery of left ventricular developed pressure (LVDP) (a), left ventricular end-diastolic pressure (LVEDP) (b), dp/dt max (c) and coronary flow (d) were examined throughout the reperfusion period. Statistical significance: $*\ P < 0.05$, $**\ P < 0.01$ vs. saline-treated group.

crossreactivity is specific to P-selectin (Mulligan et al., 1992). First, we evaluated PB1.3 in the same experimental conditions as for the evaluation conducted with sialyl Lewis X-oligosaccharide. The values for the recovery of the left ventricular functions and coronary flow after 45 min of reperfusion are summarized in Table 3. Treatment with PB1.3 at a dose of 0.6 mg/min resulted in a prominent recovery of left ventricular developed pressure 45 min after the reperfusion ($59.9 \pm 9.3\%$ vs. $30.9 \pm 6.0\%$ in the saline-treated group, $P < 0.05$). Second, we examined the time-course of left ventricular functions and coronary flow. The results are summarized in Fig. 2a–d. The recovery in left ventricular developed pressure improved steadily throughout the period of reperfusion (Fig. 2a). In the saline-treated group, left ventricular end-diastolic pressure elevated significantly to 90.3 ± 2.8 mm Hg 5 min after the reperfusion as compared with 65.3 ± 7.2 mm Hg in the PB1.3-treated group ($P < 0.01$). Moreover, left ventricular end-diastolic pressure steadily declined during the reperfusion period in hearts treated with PB1.3 compared to saline-treated hearts (Fig. 2b). Similarly, the treatment with PB1.3 significantly improved dp/dt max 45 min after the reperfusion ($60.4 \pm 9.5\%$ vs. $24.0 \pm 4.6\%$ in the saline-treated group, $P < 0.01$) and showed significant improvement throughout the reperfusion period (Fig. 2c). In addition to the left ventricular functions, the treatment with PB1.3 ameliorated the recovery of coronary flow for the early period (< 10 min) after reperfusion (5 min after the reperfusion; $38.9 \pm 5.5\%$ vs. $17.9 \pm 5.6\%$ in the saline-treated group, $P < 0.05$, 10 min after reperfusion; $55.2 \pm 9.2\%$ vs. $28.2 \pm 7.7\%$ in the saline-treated group, $P < 0.05$) (Fig. 2d).

4. Discussion

Myocardial stunning is defined as a mechanical dysfunction that persists after reperfusion despite the absence of irreversible myocardial cell death (Bolli, 1990). The

reversible left ventricular dysfunction that occurs long-term after coronary artery disease, in which ischemia-reperfusion often occurs, remains a serious clinical problem (Camici et al., 1997). In the past decade, two mechanisms have been proposed for myocardial stunning: free radical generation and calcium overload (Bolli, 1990; Williams, 1996). However, the mechanisms causing the dysfunction are not thoroughly understood. Neutrophils have been postulated as a source of oxygen radical in inflamed tissues after ischemia-reperfusion (Bolli, 1990; Williams, 1996). In the present study, we investigated the roles of selectins in the development of the neutrophil-mediated myocardial dysfunction.

Selectins are involved in the initial step in the development of the inflammatory reactions (Bevilacqua and Nelson, 1993; Tedder et al., 1995; Henricks and Nijkamp, 1998). An oligosaccharide containing sialyl Lewis X, which is a ligand for E-, P-, and L-selectins (Varki, 1997), has been used as a selectin inhibitor in various studies. Together with anti-P-selectin monoclonal antibody (PB1.3), these inhibitors have been well characterized for their specific inhibitory effects on selectin-mediated cell adhesion (Mulligan et al., 1992; Winn et al., 1993). For example, the administration of PB1.3 and sialyl Lewis X-oligosaccharide significantly attenuated myocardial necrosis after ischemia-reperfusion in various models (Lefer et al., 1994, 1996; Weyrich et al., 1993; Buerke et al., 1994). However, the pathophysiological roles of selectins in myocardial stunning have not been well characterized. Miura et al. (1996) recently reported treatment with fucoidin improved the recovery of left ventricular function in blood-perfused neonatal lamb heart. Fucoidin is a sulfated fucose oligosaccharide derived from seaweed and is known to have inhibitory effects against P- and L-selectins (Varki, 1994). In the present study, we examined the role of selectins in neutrophil-mediated left ventricular dysfunction using the well-characterized selectin inhibitor, sialyl Lewis X-oligosaccharide as well as PB1.3.

We employed an isolated rat heart reperfused with human neutrophils at a dose of 30 million cells after global ischemia for 20 min as a neutrophil-dependent left ventricular dysfunction model. We observed neutrophil-dependent systolic and diastolic dysfunction throughout the reperfusion period of 45 min, whereas coronary flow recovered within 45 min after the reperfusion (Table 1). In this experimental condition, no necrotic area was observed in the hearts assessed by the triphenyltetrazolium chloride staining (data not shown). The isolated rat heart with prolonged systolic and diastolic dysfunction in the absence of irreversible injury represents an experimental model of myocardial stunning (Moreyra et al., 1996). To evaluate the effects of selectin blockers, we chose the experimental condition in which the hearts were perfused with 30 million neutrophils. In this condition, left ventricular dysfunction was observed by the decrease of various parameters including developed pressure, end-diastolic pressure and dp/dt max (Table 1). The experimental conditions such as ischemic time, reperfusion time and count of neutrophils were well correlated to those of a previous study (Shandelya et al., 1993). First, we showed that the administration of sialyl Lewis X-oligosaccharide significantly improved the neutrophil-mediated left ventricular dysfunction after 20 min of global ischemia followed by 45 min of reperfusion (Table 2), which is consistent with a preliminary observation by Jacobson et al. (1995). Second, PB1.3 improved left ventricular dysfunction to a similar degree (Table 3, Fig. 2). These results demonstrated that selectins, particularly P-selectin, played an important role in the pathogenesis of neutrophil-mediated myocardial stunning. To our knowledge, this is the first direct demonstration that specific inhibition of P-selectin results in the inhibition of neutrophil-mediated systolic and diastolic dysfunction, in addition to myocardial necrosis and the no-reflow phenomenon that had been previously reported as P-selectin-dependent.

In the case of coronary flow, neutrophil-dependent reduction was observed in the early stage of reperfusion (< 15 min), with a return to the control level within 45 min after the reperfusion in our model (Fig. 1). It remains to be examined whether the decrease of coronary flow in the early stage is involved in the development of the left ventricular dysfunction. Recently, Ritter and McDonagh (1997) demonstrated using a similar model that neutrophils are trapped in coronary capillaries from approximately 5 min after the reperfusion. Trapped neutrophils have been implicated in the no-reflow phenomenon in which a significant proportion of capillaries fails to reperfuse upon reinstitution of the blood flow despite restoration of adequate perfusion pressure (Jerome et al., 1994). Upon interaction with endothelial cells, neutrophils are activated leading to the production of free radicals, which are considered a possible cause of myocardial stunning (Bolli, 1990; Williams, 1996). In our experiments, decrease of coronary flow preceded left ventricular dysfunction, and the failure of left ventricular was observed even after the recovery of coronary flow. In fact, in isolated rat heart perfused with human neutrophils, production of oxygen radicals in coronary artery was detected only in the presence of neutrophils (Shandelya et al., 1993). In summary, it is possible that neutrophils trapped during the early stage of reperfusion are involved in the reduction of coronary flow and in deterioration of the left ventricular function through oxygen radical formation and subsequently, the failure continues even after the recovery of coronary flow in our model. We observed that the treatment with PB1.3 improved significantly the recovery of coronary flow for 10 min after reperfusion (Fig. 2). Recently, DeLano et al. (1997) demonstrated that in vivo administration of PB1.3 significantly attenuated superoxide formation through the reduction of neutrophil rolling and adhesion in rat mesenteric artery. These results suggest that the improvement of left ventricular function induced by the selectin inhibitor seen

in the present study is likely, at least in part, due to the inhibition of adhesion and subsequent activation of neutrophils.

In conclusion, we demonstrated that P-selectin had important roles in the pathogenesis of neutrophil-mediated left ventricular dysfunction using isolated rat heart perfused with human neutrophils. These observations suggest that the inhibition of selectins is a useful therapeutic approach to enhancing the recovery of cardiac function or myocardial stunning, in addition to myocardial necrosis following ischemia-reperfusion, to which the therapeutic benefit of selectin inhibitors has been well demonstrated in experimental models.

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