Effects of a Monoclonal Antibody to P-Selectin on Recovery of Neonatal Lamb Hearts After Cold Cardioplegic Ischemia

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Background—The interaction between endothelium and leukocytes plays a crucial role in ischemia-reperfusion injury. P-selectin, which is expressed on activated endothelium, mediates the first step in leukocyte adherence to the endothelium. This study examined the effects of a monoclonal antibody (mAb) against P-selectin on the recovery of cardiac function and myocardial neutrophil infiltration after ischemia.

Methods and Results—Thirteen blood-perfused, isolated neonatal lamb hearts underwent 2 hours of hypothermic cardioplegic arrest and 2 hours of reperfusion. Immediately before reperfusion, mAb to P-selectin was administered to the perfusate (15 μg/mL) in 6 hearts (group P-sel). In control (n=7), the same volume of saline was added. Isovolumic left ventricular function and coronary blood flow were measured. At 2 hours after reperfusion, myocardial myeloperoxidase activity, an index of neutrophil accumulation, was assayed. At 30 minutes of reperfusion, hearts treated with mAb to P-selectin achieved significantly greater recovery of maximum developed pressure (70±4% in control versus 77±2% in group P-sel, $P<0.01$), maximum positive first derivative of pressure (dP/dt) (64±7% in control versus 73±5% in group P-sel, $P<0.05$), and maximum negative dP/dt (61±6% in control versus 70±6% in group P-sel, $P<0.05$) compared with control. Percent baseline of coronary blood flow was also significantly increased in group P-sel (135±40% in control versus 205±43% in group P-sel, $P<0.05$). Myocardial myeloperoxidase activity was significantly lower ($P<0.05$) in group P-sel (4.7±3.2) versus control (16.0±10.1). (Units are change in absorbance/min/g tissue.)

Conclusions—The functional blockade of P-selectin resulted in better recovery of cardiac function and attenuated neutrophil accumulation during early reperfusion. Strategies to block P-selectin mediated neutrophil adherence may have clinical application in improving myocardial function at early reperfusion. (***Circulation.* 1998;98:II-391-II-398.)**

Key Words: ischemia ■ reperfusion ■ leukocytes ■ P-selectin ■ antibodies

Leukocytes have been implicated as a major contributor to ischemia-reperfusion injury.[1,2] Activated leukocytes infiltrate tissues and release reactive oxygen species and proteolytic enzymes, such as myeloperoxidase and elastase, which cause organ dysfunction.[3,4] Also, leukocytes have been thought to plug small vessels during reperfusion and play a role in the " no reflow phenomenon."[5] Various attempts directed at the reduction of leukocyte activity or function have been shown to decrease ischemia-reperfusion injury in the heart. Leukocyte-depleted reperfusion,[6,7] monoclonal antibody to adhesion molecules on leukocytes,[8,9] or inhibitors of elastase release[10] have been demonstrated to improve cardiac function and coronary flow reserve.

Expression of P-selectin, a member of the selectin family of adhesion glycoproteins, on activated endothelial cells is an initial step in the adhesion of leukocytes to the endothelium.[11] P-selectin, located in the Weibel-Palade bodies of endothelium in the unactivated state, is rapidly translocated to the cell surface on stimulation by ischemia-reperfusion or inflammatory mediators. P-selectin interacts with P-selectin glycoprotein ligand on the neutrophil surface which mediates the rolling of leukocytes on the endothelium, followed by firm adhesion, extravasation, and infiltration into the tissue.[12]

This study examined the effect of immunoneutralization of P-selectin with a novel blocking monoclonal antibody (mAb) to ovine P-selectin on recovery of cardiac function and myocardial neutrophil infiltration after a period of hypothermic ischemia, using an isolated blood-perfused neonatal lamb heart model.[13,14]

Methods

Experimental Preparation

An isolated, blood-perfused heart model, as previously described,[6,13,14] was used to examine 13 hearts from neonatal lambs (3.4 to 5.2 kg, 2 to 8 days old). Animals were anesthetized with intramuscular ketamine (50 mg/kg), intubated, and mechanically ventilated. Animals in this study received humane care in compliance with "Principles of Laboratory Animal Care" formulated by the fNational Society for Medical Research and "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH

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Publication No. 86-23). After an intravenous injection of Fentanyl (100 μg/kg), the heart was exposed through a median sternotomy. Coronary perfusion was established with a roller pump and an oxygenator system (Bio-2, American Bentley) by placing an arterial cannula in the brachiocephalic trunk and a venous cannula in the right ventricle through the main pulmonary artery. Heparinized, fresh, homologous blood was used as the perfusate. The heart was excised while coronary perfusion was maintained by extracorporeal circulation; the heart was then placed in a temperature controlled water bath. A sampling catheter was inserted into the coronary sinus via the hemiazygos vein for coronary venous blood gas analysis (Stat Profile 5, NOVA Biomedical). A latex balloon with a pressure transducer (SPC-350, Millar Instruments Inc) was placed into the left ventricular (LV) cavity through the apex to measure LV function. Arterial pH was kept at 7.4 using pH stat strategy. Myocardial temperature was monitored by thermal probes and the perfusate was controlled at 37°C, except during the hypothermic phase. Coronary perfusion pressure was maintained at 60 mm Hg, except during the first 10 minutes of rewarming periods.

Measurements

Isovolumic contractile function of LV was measured by stepwise inflations of an intra-ventricular balloon with saline, as described previously.[7] The systolic function was evaluated by measuring the maximum developed pressure (DP), maximum positive LV first derivative (dP/dt), DP at a constant balloon volume (V10), and dP/dt at V10. V10 was defined as the balloon volume needed to produce an end-diastolic pressure (EDP) of 10 mm Hg during baseline measurement. Maximum negative dP/dt and LVEDP at V10 were measured to assess the diastolic function. These data were expressed as the percent recovery of baseline. Coronary blood flow was measured by an electromagnetic flowmeter, which was connected to the venous cannula. Coronary endothelial function was assessed by the coronary vascular resistance (CVR) response to 10^{-7} mol/L and 10^{-6} mol/L of acetylcholine and 10^{-10} mol/L and 10^{-9} mol/L of bradykinin infusion. The maximum decrease in CVR during vasodilator infusion, divided by baseline CVR, was defined as the CVR response. To assess endothelium-independent vasodilator capacity, 3×10^{-5} mol/L and 10^{-4} mol/L of trinitroglycerin was infused in a similar fashion, and the CVR response was measured. Arterial and venous blood were collected and myocardial oxygen consumption (MVO_2) was calculated using the following equation:

$$MVO_2\ (mL \cdot min^{-1} \cdot 100\ g^{-1}\ tissue) = \frac{(\text{arterial } O_2 \text{ content} - \text{coronary sinus } O_2 \text{ content}) \times CBF\ (\text{coronary blood flow})}{\text{myocardial wet weight}}$$

where O_2 content (mL/dL) = $1.36\times Hb\times O_2$ saturation/$100+0.003\times PO_2$.

Circulating white blood cell (WBC) and platelet counts were measured at baseline, 10 minutes, 60 minutes, and 120 minutes of reperfusion using an automated counter (Technicon H-1, Miles).

Myocardial tissue myeloperoxidase (MPO) activity was measured by the spectrophotometric method described by Bradley.[15] The myocardial tissue was suspended in 50 mmol/L potassium phosphate buffer (pH 6.0), containing 0.5% hexadecyltrimethylammonium bromide (Sigma Chemical) to extract the enzyme, and homogenized 4 times for 10 seconds. The samples were then sonicated, freeze-thawed 3 times with liquid nitrogen, and centrifuged at 40 000*g* for 15 minutes. The supernatant (0.1 mL) was mixed with 2.9 mL of 50 mmol/L potassium phosphate buffer (pH 6.0) containing 0.167 mg/mL *o*-dianisidine dihydrochloride (Sigma Chemical) and 0.0005% hydrogen peroxide (Sigma Chemical). The absorbance change at 460 nm was measured over 2 minutes at room temperature.

Malondialdehyde (MDA) and 4-hydroxyalkenals, which are the end products derived from the breakdown of polyunsaturated fatty acids during the process of lipid peroxidation, were measured in myocardial tissue using the spectrophotometric method.[16] Myocardial tissue was homogenized in 20 μmol/L Tris-HCl buffer at pH 7.4 and centrifuged at 3000*g* for 10 minutes at 4°C. The supernatant was collected and used for the assay (LPO-586, Calbiochem).

TABLE 1. Baseline Values

Variables	Control	mAb/P-selectin	*P*
Age (days)	4.6±1.6	4.8±1.7	NS
Body weight (kg)	4.0±0.7	4.3±0.5	NS
LV function			
Maximum DP (mm Hg)	131±16	132±14	NS
DP at V10 (mm Hg)	121±21	121±15	NS
Maximum positive dP/dt (mm Hg/s)	1972±338	1879±251	NS
Positive dP/dt at V10 (mm Hg/s)	1845±365	1656±221	NS
Maximum negative dP/dt (mm Hg/s)	1408±233	1462±129	NS
Coronary blood flow ($mL \cdot min^{-1} \cdot 100\ g^{-1}$)	270±40	242±54	NS
MVO_2 ($mL \cdot min^{-1} \cdot 100\ g^{-1}$)	3.89±2.04	2.77±1.27	NS
White blood cell count (/mm³)	2796±930	3333±651	NS
Platelet count (k/mm³)	50.5±24.0	46.8±22.5	NS

LV indicates left ventricular; DP, developed pressure; dP/dt, first derivative of pressure; V10, left ventricular volume to produce an end-diastolic of 10 mm Hg during pre-ischemic period; MVO_2, myocardial oxygen consumption.

Experimental Protocol

Baseline measurements were completed after a 15-minute equilibrium period. The perfusate and water bath were then rapidly cooled to 10°C. After 10 minutes of cooling, coronary perfusion was stopped and 20 mL/kg of cardioplegic solution was given followed by topical cooling (myocardial temperature was kept <10°C). A second dose of cardioplegia (10 mL/kg) was given after 60 minutes. The cardioplegic solution consisted of 2.5% dextrose and 0.45% NaCl solution with 20 mEq/L of potassium chloride and 6 mEq of sodium bicarbonate. After 2 hours of cardioplegic arrest, reperfusion was begun with the perfusate maintained at room temperature and then rewarmed to normothermia over 20 minutes. Mean coronary perfusion pressure was maintained at 20 mm Hg for the first 5 minutes, raised to 40 mm Hg for the next 5 minutes, and then kept at 60 mm Hg until the end of the experiment. Ninety-five percent O_2 and 5% CO_2 was used during the first 15 minutes of reperfusion. Thereafter, the gas was changed to 20% O_2, 5% CO_2, 75% N_2. After 2 hours of reperfusion, coronary perfusion was terminated. Cold (4°C) saline (20 mL/kg) was then immediately perfused into the coronary circulation. LV tissue was excised and stored at −80°C before assaying myocardial tissue MPO activity and lipid peroxidation.

In 6 experiments, murine anti-ovine P-selectin monoclonal antibody was added to the perfusion circuit 5 minutes before reperfusion to yield a final concentration of 15 μg/mL. This mAb was produced using purified ovine P-selectin from platelets as an antigen. Monoclonal antibodies were screened for selective reactivity with activated compared with unstimulated ovine platelets. mAbs were further screened for inhibition of neutrophil adhesion to platelets monolayers in static conditions and in a flow chamber. The mAb used in this study, 6F3, is of IgG 1 isotype and blocked neutrophil rolling on activated platelet monolayers in vitro (data not shown).

Statistics

All values are expressed as mean±SD. Data were compared using the 2-tail unpaired Student's *t* test or 2-way repeated measured ANOVA. $P<0.05$ was considered to be significant.

Results

Baseline Measurement

There was no significant difference between groups with regard to baseline data (Table 1).

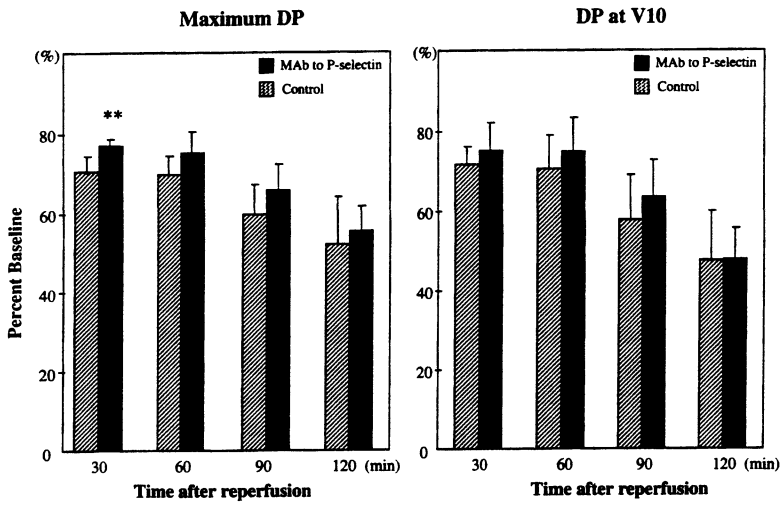

Figure 1. Percent recovery of maximum and volume-normalized (V10) LV developed pressure during reperfusion. Data expressed as mean±SD. $^{**}P<0.01$ compared with control. DP indicates developed pressure; V10, intraventricular balloon volume giving LV end-diastolic pressure of 10 mm Hg at baseline.

LV Function

Hearts treated with mAb to P-selectin achieved significantly greater recovery of both LV systolic function indices, including maximum DP (70±4% in control versus 77±2% in group P-sel, $P<0.01$; Figure 1), positive maximum dP/dt (64±7% in control versus 73±5% in group P-sel, $P<0.05$; Figure 2), and dP/dt at V10 (65±8% in control versus 75±6% in group P-sel, $P<0.05$; Figure 3), and diastolic indices, including negative maximum dP/dt (61±6% in control versus 70±6% in group P-sel, $P<0.05$) and EDP at V10 (13.3±1.0 mm Hg in control versus 11.7±0.9 mm Hg in group P-sel, $P<0.05$) at 30 minutes of reperfusion than control hearts. At 60, 90, and 120 minutes of reperfusion, there was a tendency toward better LV function recovery in anti P-selectin mAb treated group.

Coronary Blood Flow

Hearts treated with mAb to P-selectin had significantly higher coronary blood flow than the control at 30 minutes after reperfusion (135±40% of baseline in control versus 205±43% of baseline in group P-sel, $P<0.05$; Figure 4). There was no significant difference in heart rate between groups throughout the experiment (data not shown).

Myocardial Oxygen Consumption and Coronary Vascular Resistance Response

There were no significant differences in MVO_2 between groups (Table 2). Recovery of CVR response to any concentration of acetylcholine, bradykinin, or trinitroglycerin at 60 minutes of reperfusion was also not significantly different between groups (Table 3).

White Blood Cell and Platelet Count

In hearts treated with mAb to P-selectin, WBC count at 60 minutes reperfusion was significantly higher than at 10 minutes reperfusion (74.7±8.0% of baseline at 10 min of reperfusion versus 82.3±7.8% of baseline at 60 min of reperfusion $P<0.05$), which was not observed in control hearts (Figure 5). There were no significant differences in % baseline of WBC and platelet counts at individual time points during reperfusion between the 2 groups.

Myocardial MPO Activity and Lipid Peroxidation

Myocardial MPO activity at 120 minutes of reperfusion was significantly ($P<0.05$) lower in the mAb to P-selectin group than control group (4.7±3.2 ΔA/(min · g) in group P-sel and 16.1±10.1 ΔA/(min · g) in control, where ΔA/(min · g) is the change of absorbance per minute per gram myocardial

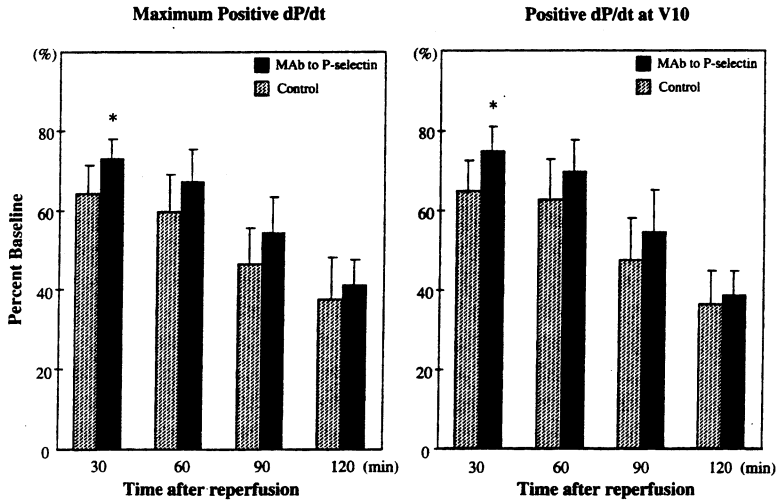

Figure 2. Percent recovery of maximum and volume-normalized (V10) LV positive first derivative of LV pressure during reperfusion. Data expressed as mean±SD. $^{*}P<0.05$ compared with control. dP/dt indicates positive first derivative of LV pressure. Other abbreviations as in Figure 1.

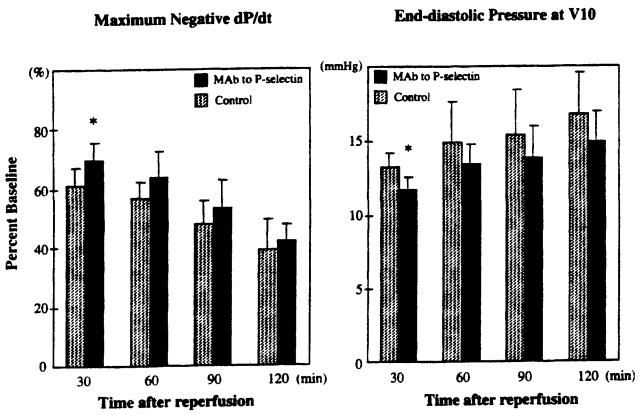

Figure 3. Percent recovery of maximum LV negative first derivative of LV pressure and V10 end-diastolic pressure during reperfusion. Data expressed as mean±SD. *$P<0.05$ compared with control.

tissue). MDA in myocardial tissue tend to be lower ($P=0.086$) in the P-selectin mAb treated group versus control group (Figure 6).

Discussion

Recent investigations have shown that interactions between leukocytes and endothelial cells are involved in reperfusion injury.[1,2] Ischemia and reperfusion activates endothelial cells to express adhesion molecules on their membrane surface. The first step of leukocyte adhesion to endothelium appears to require the expression of selectin molecules, which mediate leukocyte rolling on the endothelial cell surface.[11] On stimulation by hypoxia and inflammatory mediators, such as oxidants,[17] histamine, thrombin,[18] and terminal complement complex (C5b-9),[19] P-selectin is rapidly expressed on the endothelial cell surface membrane, providing a mechanism for tethering leukocytes to the vascular wall. A more firm adherence of leukocytes to endothelial cells requires other adhesion molecules. Interaction between leukocyte integrins of the CD11/CD18 family and their ligand, intercellular adhesion molecule-1 (ICAM-1) on endothelial cells, allows firm adhesion of leukocytes, enabling leukocyte migration

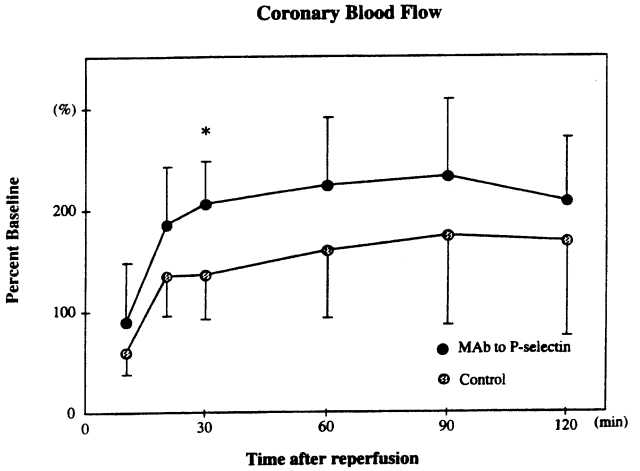

Figure 4. Percent baseline of coronary blood flow after reperfusion. Data expressed as mean±SD. *$P<0.05$ compared with control.

TABLE 2. Percent Baseline of MVO_2

Reperfusion Time (min)	Control	mAb/P-selectin	*P* value
30	102.3±36.3	125.5±57.0	NS
60	78.4±33.4	96.9±24.4	NS
90	79.2±36.9	107.1±42.6	NS
120	66.7±33.1	78.7±37.3	NS

Data are mean±SD.

across the endothelium in response to chemoattractants.[20] Extravasated leukocytes, which release free radicals and proteolytic enzymes, cause subsequent tissue injury.[21]

Anti-adhesion therapy is a new strategy to prevent ischemia-reperfusion injury; it is aimed at inhibition of the interaction between leukocytes and endothelial cells during reperfusion. Among adhesion molecules, P-selectin plays an important role in the rolling of leukocytes, which is the initial interaction between leukocytes and endothelium. It has been proposed that the rolling of a neutrophil on the endothelial cell is a necessary predecessor to a firm adhesion, which is mediated by CD11/CD18 integrins and ICAM-1 at physiological shear stress.[11] Several attempts to reduce myocardial ischemia/reperfusion injury using anti-P-selectin agents have been reported. Weyrich et al[22] demonstrated that mAb to P-selectin significantly reduced myocardial necrosis and preserved coronary vascular relaxation to acetylcholine in a normothermic ischemia/reperfusion feline heart model. Chen et al[23] also showed the protective effect of mAb to P-selectin on LV regional shortening fraction and coronary vascular endothelial function after 1 hour of normothermic ischemia followed by 1 hour of reperfusion in dogs. An additional experiment showing a therapeutic effect of species-specific mAb to P-selectin on myocardial necrosis area was reported by Tojo et al using an ischemia-reperfusion rat model.[24] The results of the present study, which was designed to simulate pediatric cardiac surgical procedures, demonstrated that immunoneutralization of P-selectin improved the recovery of both systolic and diastolic LV function as well as coronary blood flow at 30 minutes of reperfusion after 2 hours of ischemia in an isolated neonatal lamb heart model. The beneficial effects of P-selectin blockade, however, gradually declined during the course of reperfusion. The reasons for a reduced effect after early reperfusion remain speculative, but one possibility is that the role of P-selectin mediated leukocyte adherence is limited to the first 30 minutes of reperfusion. Leukocyte rolling has been reported to peak at 30 minutes of reperfusion and then return to baseline level after 60 minutes of reperfusion in the splanchnic circulation after ischemia in rats.[25] This finding is consistent with the report of Weyrich et al,[26] in which the time course of peak P-selectin expression in feline coronary venules after ischemia-reperfusion was approximately 20 minutes. They also demonstrated a gradual decline of P-selectin expression at 60, 150, and 270 minutes after reperfusion. The finding of decreased P-selectin expression in human atrial myocardium after cardiopulmonary bypass is also consistent with these experimental studies.[27] It is possible that P-selectin is rapidly expressed on the surface of endothelium and then gradually

TABLE 3. Coronary Vascular Resistance Response at Baseline and 60 Minutes of Reperfusion

	Coronary Vascular Resistance Response, %			
	Baseline		60 Min of Reperfusion	
	Control	mAb/P-selectin	Control	mAb/P-selectin
Endothelium-dependent function				
10^{-7} M of Ach	16.7±9.8	14.2±8.0	1.7±9.5	4.2±5.1
10^{-6} M of Ach	21.4±12.7	20.0±14.7	0.7±15.9	−0.8±10.0
10^{-10} M of BK	26.9±10.0	20.5±6.7	12.5±8.0	6.5±5.6
10^{-9} M of BK	34.8±14.5	29.9±9.8	14.9±10.0	8.4±7.6
Endothelium-independent function				
3×10^{-5} M of TNG	20.6±11.0	17.7±8.8	8.9±5.7	5.0±2.3
10^{-4} M of TNG	30.7±9.3	26.2±9.8	12.5±6.1	8.2±7.6

Data are mean±SD. Ach indicates acetylcholine; TNG, trinitroglycerin; and BK, bradykinin.

disappears during the early period of reperfusion. However, it has also been reported that the expression of P-selectin on the endothelial cells was sustained for 4 hours and even up to 24 hours after ischemia/reperfusion of baboon cerebral artery,[28] although P-selectin may be synthesized de novo several hours after reperfusion.

An alternative explanation for the time limitation of immunoneutralization of P-selectin is that the effect depends on an interaction between leukocytes and platelets. During reperfusion, P-selectin is also expressed on the platelet membrane surface and induces an attachment of platelets to leukocytes, resulting in an activation and transformation of leukocytes;[29] this is followed by a formation of platelet-leukocyte aggregates.[30] However, this platelet-leukocyte interaction cannot be sustained for long periods, as suggested by more recent studies. Rinder et al[31] have shown that activated whole blood caused an immediate and transient increase in leukocyte-platelet conjugates, followed by a rapid decline in the proportion of the conjugates. Moreover, it has been demonstrated that the percentage of neutrophil-platelet conjugates immediately increased at 10 minutes after the onset of cardiopulmonary bypass in patients undergoing cardiac surgery, but the percentage of these conjugates returned to normal levels during the rewarming period.[32] These findings suggest that the role of P-selectin in the interaction of leukocytes and platelets may be early and transient. Moreover, our model incorporates an extracorporeal circulation including a reservoir and an oxygenator. The artificial membrane elevates inflammatory mediators, such as complement and cytokines, and induces the expression of the other adhesion molecules, resulting in the deterioration of cardiac function. This may reduce the beneficial effect of P-selectin mAb in the late reperfusion period.

Attenuation of leukocyte adherence to endothelial cells by immunoneutralization of P-selectin has been a subject of much controversy. Kelly et al[25] reported that a mAb against P-selectin significantly attenuated the increase in neutrophil adherence to rat mesenteric venules after 60 minutes of ischemia and 2 hours of reperfusion. In contrast, Kubes et al[35] showed that a mAb against P-selectin attenuated leukocyte rolling at low flow ischemic (20% of baseline blood flow)-reperfused mesenteric venules in cats but failed to reduce the number of adherent leukocytes. Kurose et al[36] also demonstrated that mAb to P-selectin did not inhibit leukocyte adherence or emigration after ischemia (10 to 20 minutes) in mesenteric venules of rats using a intravital microscopy. Our

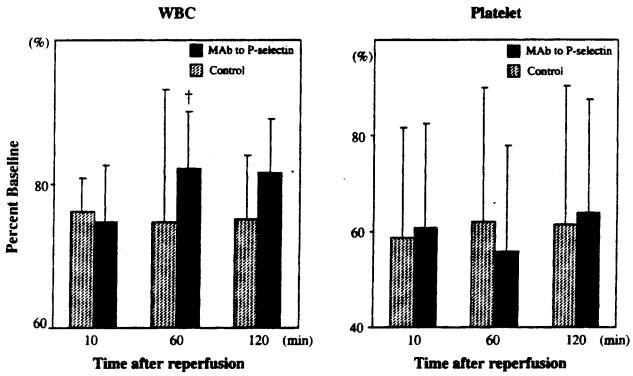

Figure 5. Percent baseline of circulating white blood cell and platelet count during reperfusion. Data expressed as mean±SD. $P<0.05$ compared with 10 minutes of reperfusion. WBC indicates white blood cell.

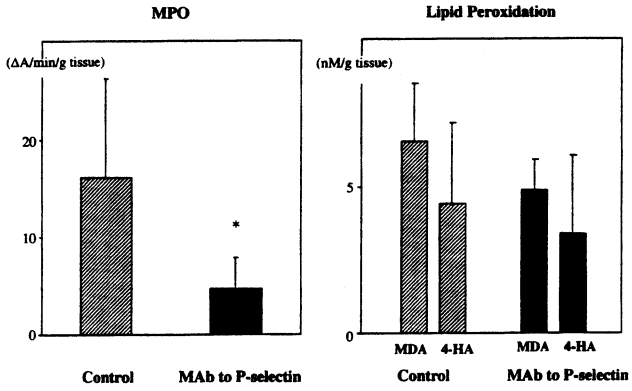

Figure 6. Myeloperoxidase activity and levels of malondialdehyde and 4-hydroxyalkenals (end products of lipid peroxidation of polyunsaturated fatty acids) in myocardial tissue at 2 hours of reperfusion. Data expressed as mean±SD. *$P<0.05$ compared with control. MPO indicates myeloperoxidase activity; ΔA/min/g tissue, the change of absorbance at 460 nm per minute per myocardial wet weight (g); MDA, malondialdehyde; and 4-HA,4-hydroxyalkenals.

findings of significantly increased circulating white blood cells at 60 minutes of reperfusion, compared with 10 minutes, and the reduction of myocardial MPO activity at 120 minutes of reperfusion in the anti-P-selectin mAb treated hearts, indirectly suggest the inhibition of adhesion and migration of leukocytes. These discrepancies regarding the protective effect on leukocyte adherence may be due to differences in experimental conditions, such as dosage of antibody, species, ischemic time, temperature, state of leukocytes (activated or inactivated), and shear stress, although there is a trend toward more protective effects of mAb to P-selectin on leukocyte adhesion with longer ischemic insults.

Other approaches for anti-selectin therapy have been reported. The ligands for selectins are carbohydrate-containing glycoproteins. The 3 known selectins, P-selectin, L-selectin, and E-selectin, can all bind to structures decorated with a sialyl-Lewis^x and a related oligosaccharide.[37] It has been demonstrated that an oligosaccharide sialyl-Lewis^x analogue inhibited selectin-mediated neutrophil adherence to endothelium in vitro.[38] Moreover, it has also been reported that this oligosaccharide significantly reduced myocardial necrosis and preserved coronary blood flow in a dog model of myocardial ischemia-reperfusion.[39] Fucoidin, which is derived from seaweed, is also an oligosaccharide (a homopolymer of sulfated fucose) that binds and functionally blocks P-selectin and L-selectin but not E-selectin.[40] A previous report from our laboratory showed that administration of fucoidin immediately before reperfusion significantly improved the recovery of LV function, oxygen consumption, and coronary blood flow.[41] A potential therapeutic advantage of using an oligosaccharides instead of a mAb in anti-selectin therapy is a shorter elimination half-life, which allows for better control of the agent's effects.

In summary, administration of a mAb to P-selectin immediately before reperfusion significantly improved both systolic and diastolic LV function and increased coronary blood flow in the early reperfusion period in blood perfused isolated neonatal lamb heart model. This mAb also attenuated migration of leukocytes into myocardial tissues. Increased understanding of the role of P-selectin, as well as other adhesion molecules, will help to elucidate the complex mechanisms regulating the interactions among leukocytes, platelets, and endothelial cells in ischemia/reperfusion injury; these insights may allow the design of myocardial protective strategies based on interference with neutrophil-endothelial interactions.

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