Adhesive Interaction Between P-Selectin and Sialyl Lewis\(^{x}\)
Plays an Important Role in Recurrent Coronary Arterial Thrombosis in Dogs

Hisao Ikeda, Takahisa Ueyama, Toyoaki Murohara, Hideo Yasukawa, Nobuya Haramaki, Hiroyuki Eguchi, Atsushi Katoh, Yoshinori Takajo, Ichiro Onitsuka, Takafumi Ueno, Shinichiro J. Tojo, Tsutomu Imaizumi

Abstract
—Cell adhesion molecules may play an important role in the disease process of acute coronary syndromes. We have shown a neutralizing anti-P-selectin monoclonal antibody and a sialyl Lewis\(^x\)-containing oligosaccharide (SLe\(^x\)-OS), an analogue of selectin ligand on leukocytes, reduce cyclic flow variations (CFVs) in a canine model of recurrent coronary arterial thrombosis, suggesting the important interaction between P-selectin and SLe\(^x\) for the pathophysiology of these syndromes. However, the functional role of these adhesion molecules in the thrombotic process remains unclear. Therefore, we investigated effects of SLe\(^x\)-OS on CFVs, platelet P-selectin expression, and morphology of the stenotic site in the same model. Anesthetized open-chest dogs (n = 34) were randomly divided into 4 groups after developing CFVs. Dogs intravenously received saline or graded doses of SLe\(^x\)-OS (5, 20, or 40 mg/kg bolus) infusion followed by a continuous infusion (5 mg·kg\(^{-1}\)·h\(^{-1}\)) for 60 minutes. By flow cytometric analysis, P-selectin expression on platelets after CFVs was significantly upregulated during CFVs. Immunohistochemical analysis revealed the incorporation of platelets with upregulated P-selectin within thrombi at the stenotic site. Microscopic observations revealed the presence of numerous platelets adhered to leukocytes at the stenotic site on the damaged endothelium. SLe\(^x\)-OS significantly reduced CFVs, inhibited the P-selectin expression on platelets, and prevented the adherence of platelets and leukocytes. These findings further support the notion that the adhesive interaction between P-selectin on platelets and SLe\(^x\) on leukocytes plays an important role in platelet-mediated thrombus formation in this model. (Arterioscler Thromb Vasc Biol. 1999;19:1083-1090.)

Key Words: thrombosis • platelets • leukocytes • P-selectin • sialyl Lewis\(^x\)

P-selectin, a member of the selectin family adhesion molecules, is an integral membrane glycoprotein located in both \(\alpha\)-granules of platelets\(^1\) and the Weibel-Palade bodies of endothelial cells.\(^2,3\) On stimulation of these cells by agonists such as thrombin,\(^4\) histamine,\(^5\) or oxygen free radicals,\(^6\) P-selectin is rapidly translocated onto the cell surface within minutes and adheres to a sialylated carbohydrate structure, sialyl Lewis\(^x\) (SLe\(^x\)), on leukocytes through a calcium-dependent mechanism.\(^7,8\) A previous study has shown that activated platelets enhance extracellular oxygen free radical generation by leukocytes through P-selectin,\(^9\) and that P-selectin mediates “rolling” of leukocytes on the endothelium\(^10\) and activated platelets.\(^11\) Furthermore, analysis of complementary DNA demonstrated the presence of a circulating form of P-selectin that possesses a deleted transmembrane segment by alternative splicing of mRNA.\(^12,13\) This soluble form of P-selectin is shown to inhibit leukocyte \(\beta\)2-integrin adhesion, and thus may protect against thrombosis and inflammatory reactions.\(^14\) Therefore, P-selectin and SLe\(^x\) are important adhesion molecules that initially mediate the cellular interaction of platelets or endothelial cells with leukocytes.

Platelet-mediated thrombus formation secondary to plaque disruption at the site of atherosclerotic lesion plays a fundamental role in the development of acute coronary syndromes including unstable angina and acute myocardial infarction.\(^15,16\) The process of these syndromes may be mediated by a complex cascade of cellular interplay among platelets, endothelial cells, and leukocytes through various adhesion molecules.\(^17,18\) Using an experimental canine model with cyclic flow variations (CFVs), which is a well established experimental canine model of recurrent coronary arterial thrombosis,\(^16,19–21\) we have recently shown that the combination of neutralizing anti-P-selectin monoclonal antibody (PB1.3) and carbohydrate analogue of SLe\(^x\) (SLe\(^x\)-OS) significantly reduces CFVs,\(^22\) suggesting that the adhesive interaction between P-selectin and SLe\(^x\) may be involved in mediation of thrombus formation in vivo. However, we had

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From the Department of Internal Medicine III (H.I., T.U., T.M., H.Y., N.H., H.E., A.K., Y.T., I.O., T.U., T.I.) Kurume University School of Medicine, Kurume, Japan; and Sumitomo Pharmaceuticals Research Center (S.J.T.), Osaka, Japan.
Correspondence to Hisao Ikeda, MD, PhD, Department of Internal Medicine III, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830, Japan. E-mail ikeikeda@med.kurume-u.ac.jp
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no evidence as to (1) whether P-selectin expression is upregulated on the surface of platelets during CFVs, (2) P-selectin expression is indeed observed within the thrombi at the stenotic site, (3) whether platelets and leukocytes adhere to the injured endothelium at the stenotic site, and (4) whether treatment with SLe^{4}-OS dose-dependently reduces CFVs and inhibits P-selectin expression on platelets during the episode of CFVs. In the present study, to further investigate the pathophysiology of the acute coronary syndromes, we tested the above issues by examining the expression of P-selectin on platelets by flow cytometry and by immunohistochemical staining in control dogs with CFVs and SLe^{4}-OS treated dogs. SLe^{4}-OS was used to investigate the functional role of the adhesive interaction between platelet P-selectin and leukocyte SLe^{4}. By electron microscopy, we also examined whether both platelets and leukocytes adhere to the coronary lumen at the stenotic site.

Methods

Surgical Preparation

All experiments were conducted in accordance with the guidelines for animal experimentation of the Kurume University School of Medicine. Healthy mongrel dogs (15 to 20 kg), anesthetized with pentobarbital sodium (30 mg/kg), were mechanically ventilated. A micromanometer was placed in the femoral artery to monitor the arterial pressure. A thoracotomy was performed in the fifth left intercostal space and the heart was suspended in the pericardial cradle. Polyethylene catheters were placed in the right atrium for drug infusion and blood sampling. A segment of the left anterior descending (LAD) coronary artery was gently dissected free from the surrounding tissue and a pulse Doppler flow probe (Hartley Instruments) was placed proximal to the constricting cylinder. Coronary blood flow (CBF) velocity was measured with a pulsed Doppler flow system (Model VF-1, Crystal Biotech). Arterial blood gases and body temperature were maintained within normal physiological ranges. Before the coronary arterial constriction, dogs were allowed to stabilize for 30 minutes. Heart rate, systolic and diastolic blood pressures, and phasic and mean CBF velocities were continuously recorded throughout the study. After baseline measurements were obtained, the endothelium of the exposed LAD coronary artery was injured by gently squeezing the artery with cushioned forceps. A cylindrical constrictor was then placed around the injured coronary artery distal to the flow probe to reduce the phasic CBF velocity to approximately 40% of the baseline level in order to eliminate reactive hyperemia after 15 seconds of temporary coronary occlusion. Subsequently, CFVs developed in 40 out of 56 dogs.

Experimental Protocol

The CBF velocity was monitored for 60 minutes to obtain the baseline value, and drugs were administered intravenously according to the following protocols. Dogs (n = 54) were divided into 4 treatment groups. The control group (n = 8) received a bolus of saline followed by a continuous infusion of saline (1 mL/h). The SLe^{4}-OS treated groups received a bolus of 5 mg/kg (n = 9), 20 mg/kg (n = 9), or 40 mg/kg (n = 9) of SLe^{4}-OS followed by a continuous infusion (5 mg·kg^{-1}·h^{-1}) for 60 minutes (the generous supply of SLe^{4}-OS was from Sumitomo Pharmaceutical, Osaka). To assess the effect of treatments, the severity of CFVs was evaluated by monitoring mean coronary blood flow (mL/min), phasic and mean nadir CBF velocities (% control), and the frequency (cycles/h) for 60 minutes before and after the treatments. CBF was determined as described previously. Briefly, CBF velocity near the center of the vessel was recorded by utilizing the pulsed Doppler principle; CBF velocity was calculated by a digital planimeter. The cross-sectional area of the vessel was approximated to an inside diameter of the Doppler flow probe, ranging in size from 2.0 to 2.5 mm. Mean CBF was then derived by multiplying mean CBF velocity by the cross-sectional area. The peak and nadir flow velocities in both phasic and mean CBF were expressed as a percentage of unconstricted CBF velocity (control). The nadir flow velocity was calculated by averaging the 3 lowest flow velocities before and after treatments. In dogs that exhibited only 2 flow restorations after the treatment, nadir flow velocity was calculated by averaging the 2.

Flow Cytometric Analysis

We first evaluated cross-reactivity of CRC81 (Biodesign International), a mouse monoclonal antibody directed against human P-selectin, to freshly isolated dog platelets. Platelet immunostaining was performed as previously described. Briefly, blood samples (2 mL) were drawn from the right atrium of 6 pentobarbital-anesthetized dogs into collecting tubes containing 7.5% EDTA. One mL of blood was stimulated with thrombin (0.5 U/mL) for 5 minutes at room temperature. A 100-μL aliquot of activated blood was immediately fixed with 1% paraformaldehyde (1 mL) for 3 hours (4°C) and platelet pellets were obtained by centrifuge at 1200g for 5 minutes at room temperature. The platelet pellets were washed with PBS containing 0.1% sodium azide (PBS/Na_{3}N), then resuspended in PBS containing 0.1% sodium azide and 2% calf serum (PBS/NaN3/CS). An additional 100-μL aliquot of blood was treated in a similar manner, without the thrombin treatment, as an inactivated control. The platelets were incubated with the primary antibody (CRC81; 5 μg/mL) or a nonspecific mouse IgG1 (0.1 mL; 5 μg/mL) for 20 minutes. The platelets were then washed in PBS/Na_{3}N to remove any excess of the unbound antibodies. A secondary antibody (0.1 mL; final concentration 5 μg/mL), FITC-conjugated goat anti-mouse IgG (TAGO Co; Cat #4349), was added to the pellet for 20 minutes. After incubation with the secondary antibody, the pellet was washed and suspended with PBS/NaN3 and then analyzed by flow cytometry (FACScan, Becton Dickinson). At least 10 000 platelets were counted, gated using the same procedure except using an anti-CD61 (glycoprotein IIb/IIIa) monoclonal antibody. The results were expressed as the percentage of specific FITC-positive platelets. Aliquots of dog platelets (n = 7) incubated with the control IgG1 were 2.6 ± 0.8% positive. In contrast, addition of thrombin (0.5 U/mL) to dog platelets increased the percent of positive cells to CRC81 (42.4 ± 4.5%). Binding of CRC81 to nontrombin activated platelets was minimal (2.2 ± 0.7%) and not significantly different from mouse IgG1 controls. Thus, CRC81 does react with thrombin-activated dog platelets. A representative histogram of the CRC81 binding to thrombin-stimulated dog platelets is shown in Figure 1.

To further examine P-selectin expression on platelets before and after CFVs, and the effect of saline (n = 5) or SLe^{4}-OS (n = 6) on P-selectin expression on platelets, flow cytometric analysis was performed as described above.

Morphological and Immunohistochemical Studies

To assess the morphological changes of the coronary arteries before and after treatment with SLe^{4}-OS, an additional 3 dogs were rendered to develop CFVs. After rapid removal of the heart, a catheter was placed into the left coronary ostium. Then, 2%
Immunohistochemical study of the coronary stenotic lesion was performed using CRC81. An additional 3 dogs were rendered to develop CFVs; the peak phasic coronary blood flow velocity decreased to 8.3 to 8.5 cycles/h and 6.6 to 6.9 mL/min, respectively (Figure 3). These values were also comparable with those reported by others.26

**Table: Hemodynamic Variables Before and After Treatments**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heart Rate (bpm)</th>
<th>Systolic</th>
<th>Diastolic</th>
<th>Peak (mm Hg) (% of Control)</th>
<th>Nadir (mm Hg) (% of Control)</th>
<th>Peak (mL/min) (% of Control)</th>
<th>Nadir (mL/min) (% of Control)</th>
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<tbody>
<tr>
<td>Control</td>
<td>125±3</td>
<td>129±5</td>
<td>101±5</td>
<td>100</td>
<td>...</td>
<td>100</td>
<td>...</td>
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<tr>
<td>Stenosis</td>
<td>124±3</td>
<td>128±5</td>
<td>100±6</td>
<td>43±1*</td>
<td>...</td>
<td>51±2*</td>
<td>...</td>
</tr>
<tr>
<td>60-min CFVs</td>
<td>121±4</td>
<td>130±5</td>
<td>103±4</td>
<td>64±3*</td>
<td>8±2</td>
<td>80±3*</td>
<td>12±3</td>
</tr>
<tr>
<td>After treatment</td>
<td>122±4</td>
<td>130±5</td>
<td>102±4</td>
<td>64±3*</td>
<td>8±2</td>
<td>80±3*</td>
<td>12±3</td>
</tr>
<tr>
<td>SLeX-OS, 5 mg/kg bolus+5 mg · kg⁻¹ · hr⁻¹, n=9</td>
<td>125±4</td>
<td>134±4</td>
<td>102±3</td>
<td>100</td>
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<td>100</td>
<td>...</td>
</tr>
<tr>
<td>Control</td>
<td>122±4</td>
<td>132±4</td>
<td>101±3</td>
<td>39±2*</td>
<td>...</td>
<td>48±2*</td>
<td>...</td>
</tr>
<tr>
<td>Stenosis</td>
<td>122±4</td>
<td>134±4</td>
<td>102±4</td>
<td>65±5*</td>
<td>8±2</td>
<td>80±4*</td>
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<td>After treatment</td>
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<td>102±3</td>
<td>65±5*</td>
<td>8±2</td>
<td>81±4*</td>
<td>13±4</td>
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<td>SLeX-OS, 20 mg/kg+5 mg · kg⁻¹ · hr⁻¹, n=8</td>
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<td>131±3</td>
<td>102±3</td>
<td>100</td>
<td>...</td>
<td>100</td>
<td>...</td>
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<tr>
<td>Control</td>
<td>127±3</td>
<td>132±3</td>
<td>102±2</td>
<td>41±3*</td>
<td>...</td>
<td>51±3*</td>
<td>...</td>
</tr>
<tr>
<td>Stenosis</td>
<td>126±4</td>
<td>131±3</td>
<td>101±2</td>
<td>65±3*</td>
<td>7±2</td>
<td>80±4*</td>
<td>11±2</td>
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<tr>
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<tr>
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<td>132±4</td>
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<td>11±3</td>
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<td>100±3</td>
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<td>19±4†</td>
<td>70±5*</td>
<td>29±6†</td>
</tr>
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</table>

Values are expressed as mean±SEM. bpm indicates beats per minutes; phasic flow, peak phasic coronary blood flow velocity; mean flow, mean coronary blood flow velocity (both phasic flow and mean flow are expressed as a percentage of unconstricted blood flow velocity, respectively); peak, average high flow during cyclic flow variations (CFVs); nadir, average low flow during CFVs; control, time period before constrictor placement; stenosis, time period after constrictor placement and before development of CFVs; 60-min CFVs, 60 minutes observation of CFVs before treatment; after treatment, observation between 0 and 60 minutes after treatment.

*P<0.05 compared with control; †P<0.05 compared with 60-min CFVs.

gluteraldehyde in PBS was perfused through the catheter at 100 mm Hg pressure for 10 minutes. The LAD coronary arteries were carefully dissected and the constrictor was removed. The segments were longitudinally dissected and visualized. The specimens were incubated in the same fixation buffer for 2 hours, and rinsed with 0.1 mol/L cacodylated buffer containing 0.1 mol/L sucrose for 12 hours. The specimens were immersed in 1% osmium tetroxide for 1 hour, dehydrated in a series of graded concentrations of cold ethanol, dried by the critical-point drying method, mounted on silver blocks, coated with about 10 nm of gold, and observed under a scanning electron microscope (S-800, Hitachi) at 20 KV.

The hemodynamic data from the 4 treatment groups of dogs are shown in the Table, and Figures 2 and 3.

**Results**

The hemodynamic data from the 4 treatment groups of dogs are shown in the Table, and Figures 2 and 3.

**Before Development of CFVs (Stenosis in Table)**

Endothelial injury and coronary constriction decreased the averaged peak phasic CBF velocity to 39% to 43% of baseline (control) and mean CBF velocity to 48% to 51% of baseline (control). Heart rate, aortic pressure, and peak phasic and mean flow velocities were comparable among the 4 groups. These data are consistent with those reported by others.26

**After Developing CFVs and Before Treatment (60-minute CFVs in Table)**

No significant changes were observed in heart rates or systolic and diastolic aortic blood pressures after the development of CFVs. The peak phasic and mean CBF velocities were similarly decreased among the 4 groups. The phasic coronary nadir flow velocity was decreased to 7% to 8% of control and mean coronary nadir flow velocity decreased to 11% to 12% of control; these values were not significantly different among the groups. The frequency and the mean CBF of CFVs was 8.3 to 8.5 cycles/h and 6.6 to 6.9 mL/min, respectively (Figure 3). These values were also comparable
among the groups. Thus, the severity of CFVs was comparable among the groups. These data are in agreement with those reported by others²⁶ and us.²²

Effects of Treatments on CFVs (After Treatment in Table)
The effects of saline and SLe⁴-OS on hemodynamics and severity of CFVs are shown in Table, and Figures 2 and 3. There were no significant effects of treatments on heart rate and aortic pressure in the 4 groups. Treatment with saline or SLe⁴-OS (a bolus of 5 mg/kg) did not cause a significant change in the nadir CBF velocity, nor the frequency or the mean CBF of CFVs. Treatment with SLe⁴-OS (a bolus of 20 mg/kg) did not increase the nadir CBF velocity (Table), but significantly decreased the frequency of CFVs \( (P<0.05) \) and significantly increased the mean CBF \( (P<0.05) \). Treatment with SLe⁴-OS (a bolus of 40 mg/kg) significantly increased the nadir CBF velocity \( (P<0.05) \) and the mean CBF \( (P<0.05) \), and significantly decreased the frequency of CFVs \( (P<0.05) \). Thus, there were dose-dependent effects of SLe⁴-OS on CFVs.

Expression of P-Selectin on Platelets and Immunohistochemical Localization of P-selectin in Thrombi
Flow cytometric detection of bound CRC81, a monoclonal antibody against P-selectin during the episode of CFVs is shown in Figure 4. The upper panel demonstrates the expression of P-selectin on platelets in a control dog with CFVs, and the lower panel in a SLe⁴-OS treated dog. Summarized data are shown in Figure 5. The P-selectin expressions on platelets before treatments (baseline and stenosis) were similar between the saline and SLe⁴-OS treated groups. After development of CFVs, the extent of P-selectin expression similarly and significantly increased in both groups \( (P<0.05) \). Although treatment with saline did not affect P-selectin expression, treatment with SLe⁴-OS significantly decreased P-selectin expression \( (P<0.05) \). The immunohistochemical staining of P-selectin within thrombi at the stenotic site was intense, but the expression of P-selectin on the damaged endothelium was undetectable (Figure 6).

Morphology of Coronary Arteries
Scanning electron photomicrograms are illustrated in Figure 7. Figure 7A demonstrates the left circumflex coronary artery with the intact endothelium. Numerous platelets and leukocytes were observed at the stenotic site with the mechanically damaged endothelium (Figure 7B). After treatment with
Leukocytes no longer attached and a few platelets were observed at the stenotic site (Figure 7C).

**Discussion**

In the present study, the important findings were as follows: (1) The surface expression of P-selectin on platelets was significantly upregulated after developing CFVs. (2) Immunohistochemical analysis showed the upregulated P-selectin expression within thrombi at the coronary stenotic site with the damaged endothelium. (3) Microscopic observations revealed the presence of numerous platelets with leukocytes at the stenotic site. (4) Treatments with SLε-OS, a unique soluble carbohydrate analogue of SLε, significantly reduced CFVs in a dose-dependent manner, suppressed P-selectin expression of platelets, and inhibited the attachment of platelets and leukocytes on the damaged endothelium. Thus, these immunohistochemical and morphological findings suggest that the adhesive interaction between P-selectin on platelets and SLε on leukocytes plays an important role in mediating platelet-mediated thrombus formation in the stenosed and endothelium-injured canine coronary arteries.

We have previously reported that conscious dogs with CFVs manifest a pathophysiology similar to acute coronary syndromes including unstable angina, acute myocardial infarction, and ischemic sudden death in humans. Episodes of CFVs have been observed during coronary angioplasty in some patients with unstable angina. As originally described by Folts et al., CFVs are caused by brief and repeated episodes of coronary occlusion secondary to focal platelet aggregation and subsequent dislodgment at the stenotic site of the coronary artery. In the present study, we used this canine model of coronary arterial thrombosis to examine the role of the adhesion molecules in the thrombotic process in vivo. We have reported that soluble P-selectin is increased in blood in patients with acute coronary syndrome, and that the combination of neutralizing anti-P-selectin monoclonal antibody (PB1.3) and carbohydrate analogue of SLε (SLε-OS) significantly reduces CFVs. These results suggest that the adhesive interaction between P-selectin and SLε may be involved in thrombus formation in vivo. In the present study, we demonstrated not only the upregulation of platelet P-selectin expression after developing CFVs by flow cytometric analysis, but also the incorporation of platelets with upregulated P-selectin expression into thrombi at the coronary stenotic site. Moreover, in the present study, scanning electron micrographic observations revealed the presence of numerous platelets with leukocytes on the damaged endothelium at the stenotic site. Our findings are additive to the results of previous clinical studies demonstrating the upregulation of platelet P-selectin expression in patients with acute coronary syndromes, subacute occlusive coronary stent thrombosis, and primary antiphospholipid syndrome, and are consistent with the results of a previous experimental study.
study showing positive immunostaining for P-selectin within the thrombus in a primate model of femoral arterial thrombosis. These findings further support the functional role of platelet P-selectin interaction with leukocyte SLe⁴ during thrombus formation of CFVs in this model.

To further investigate the role of adhesive interaction between P-selectin and SLe⁴ in the thrombotic process of CFVs, effects of SLe⁴-OS an unique soluble carbohydrate analogue of SLe⁴, were examined on CFVs and platelet P-selectin expression. SLe⁴ functions as a ligand for selectins. Soluble SLe⁴-OS is thus assumed to compete with native SLe⁴ expressed on leukocytes and inhibit the interaction between platelets and leukocytes. Recently, we have shown that a high dose of SLe⁴-OS (40 mg/kg) significantly reduced CFVs in dogs. However, the dose-dependent effects of SLe⁴-OS on CFVs were not examined in our previous study. In the present study, SLe⁴-OS reduced CFVs in a dose-dependent manner and decreased the expression of P-selectin on platelets. Moreover, the scanning electronmicrogram demonstrated that SLe⁴-OS prevented attachments of leukocytes and platelets onto the damaged endothelium. Thus, these findings suggest that blocking the function of leukocyte SLe⁴ by SLe⁴-OS downregulates the expression of P-selectin on platelets, inhibits the interaction among platelets, leukocytes and the endothelium, and thus prevents the formation of thrombi at the stenotic site.

Coronary thrombus formation at the site of culprit lesion plays an important role in the development of acute coronary syndromes including unstable angina and acute myocardial infarction. The process of these syndromes may be mediated by a complex cascade of cellular interplay among platelets, endothelial cells, and leukocytes through various adhesion molecules such as integrins, selectins, and immunoglobulin superfamilies. Among these adhesion molecules, P-selectin is rapidly translocated to cell surfaces on platelets or endothelial cells and adheres to a sialylated fucosylated carbohydrate structure such as SLe⁴ on leukocytes, when these cells are activated by thrombin or oxygen free radicals. Previous studies have indicated that activated platelets enhance extracellular oxygen free radical generation by leukocytes through P-selectin, and that P-selectin mediates “rolling” of leukocytes on the endothelium and activated platelets. In animal models of myocardial reperfusion injury, either monoclonal antibody to P-selectin or SLe⁴-OS protected against reperfusion-induced endothelial and myocardial injuries. Thus, both in vitro and in vivo experimental studies have shown that the adhesive interaction between P-selectin and SLe⁴ may have an active role in modulating vascular and tissue injuries. However, the functional role of P-selectin in the coronary thrombus formation has been fully unknown in vivo. In the previous study, Toombs et al demonstrated that occlusive thrombi formed in the presence of P-selectin antagonism lyse more rapidly in the presence of pharmacological thrombolysis in a primate model of electrically induced femoral arterial thrombosis, which is characterized by persistent fibrin-rich, platelet-poor thrombi. These results are suggestive of the role of P-selectin in stabilizing fibrin-rich, platelet-poor thrombi. The present model of cyclic flow variations is characterized by recurrent platelet-rich thrombi. Thus, the present study focused on the role of P-selectin in platelet adhesion and aggregation in vivo. Based on the results from this and the previous study, the adhesive interaction between P-selectin on platelets and SLe⁴ on leukocytes is shown to be an important regulator of not only platelet adhesion and aggregation but also the cascade of coagulation and fibrinolysis in vivo.

The present study may provide important clinical implications. Increases in the surface expression of P-selectin on...
platelets have been shown in patients with acute coronary syndromes. Recently, we have shown that leukocyte adherence to the endothelium via P-selectin plays an important role in injuries of the endothelium of the coronary artery distal to the thrombotic site in vivo in dogs, and that treatment with SLε4-OS prevented endothelial injuries distal to the thrombotic site. Taken together with the findings of the present study, it is likely that SLε4-OS not only prevents thrombus formation at the stenotic site but also preserves endothelial function of the artery distal to the thrombotic site. Because the small sugar moiety of SLε4-OS has low antigenicity when used in vivo and has a potential efficacy with oral administration, SLε4-OS could become an attractive therapeutic modality of acute coronary syndromes in humans.

The present study may have some limitations. We did not examine the expression of P-selectin on platelets after small doses of SLε4-OS, and thus the exact relationship between the expression of P-selectin on platelets and the severity of CFVs was undetermined. We did not examine which mechanisms induce the P-selectin expression on platelets in the current model. P-selectin is rapidly translocated onto the cell surface after stimulation with thrombin and/or oxygen free radicals. Thrombin and oxygen free radicals are important mediators of CFVs. Accordingly, it is possible that these agonists are generated by mechanical manipulations (endothelial damage and constriction) and induce P-selectin expression on platelets in vivo.

In conclusion, the present study is the first demonstration that the adhesive interaction between P-selectin on platelets and SLε4 on leukocytes plays an important role in platelet-mediated thrombus formation in stenosed and endothelium-injured canine coronary arteries in vivo. SLε4-OS as used in this study may provide the salutary effects against acute thrombotic events in the coronary artery in vivo.

References


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