Platelet-Derived 12-Hydroxyeicosatetraenoic Acid Plays an Important Role in Mediating Canine Coronary Thrombosis by Regulating Platelet Glycoprotein IIb/IIIa Activation

Atsushi Katoh, MD; Hisao Ikeda, MD; Toyoaki Murohara, MD; Nobuya Haramaki, MD; Hideki Ito, MS; Tsutomu Imaizumi, MD

Background—In the thrombotic process of acute coronary syndromes, the pathophysiological role of thromboxane A₂ via cyclooxygenase is well established; however, the role of 12-HETE via 12-lipoxygenase is little known. Therefore, we used OPC-29030, a novel specific inhibitor of 12-HETE synthesis, to test whether platelet-derived 12-HETE is involved in mediating cyclic flow variations (CFVs) and platelet aggregation in stenosed and endothelium-injured canine coronary arteries.

Methods and Results—After developing CFVs, dogs received a vehicle or OPC-29030 intravenously. Plasma and intraplatelet 12-HETE levels increased after CFVs. OPC-29030 but not vehicle reduced CFVs, which was associated with decreases in plasma and intraplatelet 12-HETE levels. Cessation of OPC-29030 restored CFVs in association with increases in plasma and intraplatelet 12-HETE levels. ADP and U46619 induced ex vivo platelet 12-HETE production and aggregation. After OPC-29030 administration, the ADP- and U46619-induced increases in ex vivo platelet 12-HETE production and aggregation were inhibited significantly. Platelet aggregation was linearly correlated with platelet 12-HETE production. OPC-29030 suppressed activation of human platelet glycoprotein IIb/IIIa.

Conclusions—OPC-29030 reduced intraplatelet 12-HETE levels, resulting in the inhibition of coronary thrombosis in vivo in dogs. OPC-29030 inhibited human platelet glycoprotein IIb/IIIa activation in vitro. Thus, platelet-derived 12-HETE may play an important role in mediating thrombotic process. (Circulation. 1998;98:2891-2898.)

Key Words: platelets • hydroxyeicosatetraenoic acid • platelet aggregation • glycoproteins • thrombosis

Plaque disruption with subsequent thrombus formation has been implicated as a major pathogenic mechanism for the acute coronary syndromes, including unstable angina and acute myocardial infarction. In clinical studies, coronary angiographic observations have shown the presence of platelet thrombi in these syndromes. Experimental studies have also demonstrated that platelet-mediated coronary thrombosis produces pathophysiological manifestations similar to these syndromes. Thus, acute vascular injury at the site of coronary atherosclerotic plaque substantially facilitates thrombus formation through a complex cascade of cellular interactions between platelets and vascular endothelial cells.

Metabolites of arachidonic acid play an important role in mediating thrombus formation and in regulating vascular tone. Arachidonic acid, which increases dramatically via action of the phospholipase A₂-mediated phospholipid hydrolysis, is metabolized through 2 major catalytic pathways mediated by 2 different enzymes in platelets. One is cyclooxygenase, which produces thromboxane A₂ (TXA₂), and the other is 12-lipoxygenase, which produces 12-hydroxyeicosatetraenoic acid (12-HETE), which is rapidly converted to 12-hydroxyeicosatetraenoic acid (12-HETE). In experimental and clinical studies, the pathophysiological role of TXA₂ in platelets is well established in thrombus formation at the culprit lesion of the coronary artery. However, it is still unknown whether 12-HETE plays an active role in mediating thrombus formation in vivo, although 12-HETE has been shown to potentiate platelet aggregation in vitro.

Accordingly, to further investigate the pathophysiology of the acute coronary syndromes, we tested our hypothesis that platelet-derived 12-HETE is involved in mediating thrombus formation in vivo by use of a well-established experimental canine model. 13-15 13-15 (5)-(+)-6-[3-(1-o-tolylimidazol-2-yl)sulfinylpropoxy]-3,4-dihydro-2(1H)-quinolinone (OPC-29030) is a newly developed specific inhibitor of 12-HETE synthesis in platelets (Figure 1), and its efficacy has been established in vivo. 16 Therefore, OPC-29030 was used to test...
our hypothesis in dogs. We also examined whether OPC-29030 affects plasma and intraplatelet levels of 12-HETE and thromboxane B2 (TXB2) in vivo, inhibits ex vivo platelet aggregation and 12-HETE production induced by ADP and U46619, and inhibits human platelet glycoprotein (GP) IIb/ IIIa activation in vitro.

Methods

Surgical Preparation and Experimental Protocol

All experimental protocols were conducted in accordance with the guidelines for animal experimentation issued from the Human and Animal Research Committee of Kurume University School of Medicine. Our canine model of cyclic flow variations (CFVs) was prepared as described previously.15,17 CFVs developed in 40 of 51 dogs. The remaining 11 dogs were excluded from this protocol.

Dogs (n = 40) were divided into 4 groups. After 40 minutes of stabilization of CFVs, drugs were administered intravenously. Group 1 (n = 12) received a continuous infusion of the vehicle as control (dimethylformamide; Wako Co) at 100 μg · kg⁻¹ · min⁻¹ for 40 minutes. Groups 2 (n = 4), 3 (n = 18), and 4 (n = 6) received a continuous infusion of OPC-29030 (3, 10, and 20 μg · kg⁻¹ · min⁻¹, respectively) (Otsuka Co) for 40 minutes. All dogs were monitored for an additional 40 minutes after cessation of the treatment. To assess the effects of each treatment, the severity of CFVs was evaluated by their frequency over a period of 40 minutes (cycles/40 min) before, during, and after cessation of the treatment. The severity of CFVs was also evaluated by their coronary blood flow (CBF) velocity (% control) as previously described.15

Measurements of Plasma and Intraplatelet 12-HETE and TXB2 Levels In Vivo

In groups 1 (n = 10) and 3 (n = 12), to examine the effects of OPC-29030 on plasma and intraplatelet levels of 12-HETE and thromboxane B2 (TXB2), 4 groups were divided into 4 groups. After 40 minutes of stabilization of CFVs, drugs were administered intravenously. Group 1 (n = 12) received a continuous infusion of the vehicle as control (dimethylformamide; Wako Co) at 100 μg · kg⁻¹ · min⁻¹ for 40 minutes. Groups 2 (n = 4), 3 (n = 18), and 4 (n = 6) received a continuous infusion of OPC-29030 (3, 10, and 20 μg · kg⁻¹ · min⁻¹, respectively) (Otsuka Co) for 40 minutes. All dogs were monitored for an additional 40 minutes after cessation of the treatment. To assess the effects of each treatment, the severity of CFVs was evaluated by their frequency over a period of 40 minutes (cycles/40 min) before, during, and after cessation of the treatment. The severity of CFVs was also evaluated by their coronary blood flow (CBF) velocity (% control) as previously described.15

Measurements of Plasma OPC-29030 Levels

In group 3 (n = 12), plasma OPC-29030 levels were measured during and after the OPC-29030 treatment. The measurements were performed with high-performance liquid chromatography as previously described.15

Measurements of Intraplatelet cAMP Levels

In group 3 (n = 10), to examine the effects of OPC-29030 on intraplatelet cAMP levels, blood was collected into a prechilled siliconized glass tube containing EDTA and isobutylmethylxanthine 0.5 mmol/L (Sigma) and was kept at 4°C. The platelet pellet was obtained as described above. The residual pellet was resuspended in modified HEPES–Tyrode’s buffer without Ca²⁺ or Mg²⁺ and containing EDTA and isobutylmethylxanthine. The platelets were washed twice with the same buffer, and the number of platelets was adjusted to 100 000/mm³. Measurements of intraplatelet cAMP levels were performed in duplicate with a commercially available competitive enzyme immunoassay kit (Biotrak, Amersham Pharma- cia Biotech).

Analysis of Human Platelet GP IIb/IIIa Activation

With peripheral blood collected from 5 healthy volunteers, washed platelets were prepared according to the previously described method and incubated at 22°C for 10 minutes with 300 ng/mL OPC-29030, 1 μmol/L baicalein, or buffer. Then, the samples were incubated with a saturating concentration of the FITC-conjugated monoclonal antibody PAC-1 (Becton-Dickinson), followed by incubation at 22°C for 10 minutes with ADP at 0.05 to 5 μmol/L, U46619 at 0.05 to 5 μmol/L, thrombin at 5 to 500 mU/mL, or buffer. The PAC-1 antibody has a specificity for an epitope that is exposed on the fibrinogen receptor GP Ib/IIIa only after an activation-dependent conformational change.13 The samples were then fixed in paraformaldehyde 1% at 22°C for 30 minutes and were analyzed within 2 hours by flow cytometry (Becton-Dickinson). Background binding, obtained from parallel samples run with FITC-conjugated immunoglobulin M (Pharmingen) was subtracted from each test sample. PAC-1 binding in the presence of maximal thrombin (500 mU/mL) and buffer alone was assigned to be 100 U fluorescence.

Statistical Analysis

Values are presented as mean±SEM. Statistical comparisons between groups were performed with a paired Student’s t test. Multiple comparisons were analyzed by repeated-measures ANOVA. The relationship between 2 parameters was analyzed by use of a linear regression analysis. Differences were considered statistically significant at a value of P<0.05.
Ashton et al.17 Heart rate, aortic pressure, and peak phasic and decreased the averaged peak phasic CBF velocity to 35% to
Endothelial injury and coronary constriction significantly
Before Development of CFVs
Hemodynamics

### Hemodynamic Variables Before, During, and After Treatments

<table>
<thead>
<tr>
<th>Group 1 (dimethylformamide, n=12)</th>
<th>Heart Rate, bpm</th>
<th>Aortic Pressure, mm Hg</th>
<th>Phasic Flow Velocity, % of Control</th>
<th>Mean Flow Velocity, % of Control</th>
<th>Frequency, CFVs/40 min</th>
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<tr>
<td>Baseline</td>
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<td>132±6</td>
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<td>102±6</td>
<td>37±1*</td>
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<td>103±6</td>
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<td>135±7</td>
<td>104±3</td>
<td>63±7*</td>
<td>14±2</td>
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<tr>
<td>After cessation of treatment</td>
<td>121±5</td>
<td>133±5</td>
<td>105±5</td>
<td>63±5*</td>
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<th>Group 2 (OPC-29030 3 µg · kg⁻¹ · min⁻¹, n=4)</th>
<th>Heart Rate, bpm</th>
<th>Aortic Pressure, mm Hg</th>
<th>Phasic Flow Velocity, % of Control</th>
<th>Mean Flow Velocity, % of Control</th>
<th>Frequency, CFVs/40 min</th>
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<tr>
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<td>Stenosis</td>
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<td>102±8</td>
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<td>135±10</td>
<td>101±9</td>
<td>70±9*</td>
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<th>Group 3 (OPC-29030 10 µg · kg⁻¹ · min⁻¹, n=18)</th>
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<th>Aortic Pressure, mm Hg</th>
<th>Phasic Flow Velocity, % of Control</th>
<th>Mean Flow Velocity, % of Control</th>
<th>Frequency, CFVs/40 min</th>
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<tr>
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<th>Aortic Pressure, mm Hg</th>
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<th>Mean Flow Velocity, % of Control</th>
<th>Frequency, CFVs/40 min</th>
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</tr>
<tr>
<td>Initial CFVs</td>
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<td>136±9</td>
<td>99±8</td>
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<td>81±9†</td>
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<td>37±7†</td>
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<td>After cessation of treatment</td>
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<td>126±11</td>
<td>88±9</td>
<td>59±5*</td>
<td>29±9†</td>
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Phasic flow indicates peak phasic CBF velocity; mean flow, mean CBF velocity (both phasic and mean flow are expressed as percent of unconstricted CBF velocity); peak, average high flow during CFVs; nadir, average low flow during CFVs; frequency, frequency of CFVs/40 min; baseline, time period before constrictor placement; stenosis, time period after constrictor placement and before development of CFVs; initial CFVs, 40-min observation of CFVs before treatment; during treatment, 40-min observation during treatment; and after cessation of treatment, 40-min observation after cessation of treatment. Values are expressed as mean±SEM.

*P<0.05 vs baseline; †P<0.05 vs initial CFVs; ‡P<0.05 vs during treatment.

### Results

**Hemodynamics**

**Before Development of CFVs**
Endothelial injury and coronary constriction significantly decreased the averaged peak phasic CBF velocity to 35% to 38% of baseline and the mean CBF velocity to 47% to 52% of baseline. These figures are similar to those reported by Ashton et al.17 Heart rate, aortic pressure, and peak phasic and mean CBF velocities were comparable among the 4 groups (Table).

**Initial CFVs**
No significant changes were observed in heart rate or in systolic and diastolic aortic pressures after development of CFVs. The peak phasic and mean CBF velocities were decreased similarly among the groups. The phasic CBF velocity was decreased to 12% to 14% of baseline and mean nadir CBF velocity to 11% to 14% of baseline. The averaged frequency of CFVs was 4.1 to 4.3 cycles/40 min. These values were also similar among the 4 groups. Thus, the severity of CFVs before the treatment was similar among the 4 groups. These figures were similar to those reported by Ashton et al.17 and us5,13–15 (Table).

**During Treatment**
The treatments did not cause significant changes in heart rate or aortic pressure in groups 1, 2, and 3, but they did in group 4. In groups 1 and 2, the treatment did not cause significant changes in the nadir CBF velocity or the frequency of CFVs. In group 3, OPC-29030 significantly increased the nadir CBF velocity and significantly decreased the frequency of CFVs in association with increases in plasma OPC-29030 levels (212±30 ng/mL at 20 minutes and 286±48 ng/mL at 40 minutes) (Figure 2, Table).

**After Cessation of Treatment**
In groups 1, 2, and 3, cessation of treatment did not cause significant changes in heart rate or aortic pressure. In group 4, cessation of treatment decreased heart rate and increased aortic pressure, although statistically insignificantly. In groups 1 and 2, cessation of treatment did not cause significant changes in either nadir CBF velocity or frequency of CFVs. In group 3, cessation of OPC-29030
treatment returned the nadir CBF velocity and the frequency of CFVs to the levels before treatment. Plasma OPC-29030 levels after cessation of treatment decreased to 68±6 and 31±8 ng/mL at 20 and 40 minutes, respectively (Figure 2). In group 4, cessation of the OPC-29030 treatment significantly restored CFVs (Table).

Plasma and Intraplatelet 12-HETE and TXB$_2$ Levels

In group 1 (Figure 3A), plasma 12-HETE levels after CFVs were significantly increased compared with those before CFVs. Neither treatment nor cessation of treatment caused significant changes in plasma 12-HETE levels. In group 3 (Figure 3B), plasma 12-HETE levels after CFVs were significantly increased compared with those before CFVs. OPC-29030 significantly decreased plasma 12-HETE levels. After cessation of treatment, plasma 12-HETE levels were restored to those observed during initial CFVs. In group 3, intraplatelet 12-HETE levels changed to the same degree as plasma levels (Figure 4).

In group 3, plasma and intraplatelet TXB$_2$ levels were not changed by OPC-29030 treatment (plasma TXB$_2$, 0.91±0.14 ng/mL during CFVs versus 0.99±0.21 ng/mL during treatment, $P=\text{NS}$; intraplatelet TXB$_2$, 0.22±0.05 ng/mL during CFVs versus 0.24±0.05 ng/mL during treatment, $P=\text{NS}$).

Platelet 12-HETE and Coronary Arterial Thrombosis
Effects of Treatments on Ex Vivo Platelet Aggregation and 12-HETE Production

In group 1, both ADP and U46619 caused comparable concentration-dependent platelet aggregation before and after treatment (Figure 5A). In group 3, both agonist-induced platelet aggregation (Figure 5B) and platelet 12-HETE production (Figure 6) were significantly suppressed by OPC-29030 compared with those before treatment. There were significant linear relationships between platelet aggregation and 12-HETE production after stimulation with both agonists (Figure 7).

Intraplatelet cAMP Levels

The intraplatelet cAMP levels did not change at any given time point (Figure 8).

Effects of OPC-29030 and Baicalein on Human Platelet GP IIb/IIIa Activation

As shown in Figure 9, ADP, U46619, and thrombin caused dose-dependent platelet GP IIb/IIIa activation. OPC-29030 and baicalein significantly inhibited the platelet GP IIb/IIIa activation induced by all these agonists.

Discussion

As originally described by Folts et al., focal platelet aggregation and subsequent dislodgment at the coronary stenotic site result in brief and repeated episodes of coronary occlusion that are characterized by CFVs. The present model is now established as an in vivo model of coronary arterial thrombosis. We previously reported that conscious dogs

Effects of OPC-29030 and Baicalein on Human Platelet GP IIb/IIIa Activation

As shown in Figure 9, ADP, U46619, and thrombin caused dose-dependent platelet GP IIb/IIIa activation. OPC-29030 and baicalein significantly inhibited the platelet GP IIb/IIIa activation induced by all these agonists.
with CFVs manifest a pathophysiology similar to that of the acute coronary syndromes in humans. The major findings of the present study were that (1) there were increases in both plasma and intraplatelet 12-HETE levels after development of CFVs; (2) OPC-29030 reduced CFVs in association with decreases in both plasma and intraplatelet 12-HETE levels, whereas the cessation of OPC-29030 restored CFVs in association with increases in both plasma and intraplatelet 12-HETE levels; (3) OPC-29030 inhibited ex vivo platelet aggregation and 12-HETE production induced by ADP and U46619; and (4) OPC-29030 inhibited agonist-induced activation of human platelet GP IIb/IIIa in vitro. Thus, our results suggest that platelet-derived 12-HETE is critically involved in mediating platelet aggregation and CFVs in vivo in stenosed and endothelium-injured coronary arteries.

In the present study, OPC-29030 at 3 μg · kg⁻¹ · min⁻¹ did not affect CFVs, but OPC-29030 at 20 μg · kg⁻¹ · min⁻¹ significantly reduced CFVs, although this dosage caused systemic hemodynamic changes. Thus, decreases in CFVs by OPC-29030 at 20 μg · kg⁻¹ · min⁻¹ might not be attributable solely to its effects on platelets. In contrast, OPC-29030 at 10 μg · kg⁻¹ · min⁻¹ significantly reduced CFVs without systemic hemodynamic changes. Therefore, the dosage of 10 μg · kg⁻¹ · min⁻¹ was used for the subsequent protocols. The cessation of OPC-29030 treatment significantly restored CFVs. Our data therefore indicate that OPC-29030 reduces CFVs by its antiplatelet effects. To further investigate the role of 12-HETE in the pathogenesis of the thrombotic process of CFVs, we measured plasma and intraplatelet 12-HETE levels. In this connection, a previous in vitro study showed that 12-HETE was produced by aggregated platelets, and a previous study using the same dog model with CFVs demonstrated that the 12-HETE synthesis was increased by 10-fold in the stenosed and endothelium-injured coronary arteries. In this study, both plasma and intraplatelet 12-HETE levels during CFVs increased significantly compared with those before CFVs. The inhibitory effects of OPC-29030 on CFVs were associated with decreases in both plasma and intraplatelet 12-HETE levels. The IC₅₀ of OPC-29030 for ex vivo platelet 12-HETE inhibition was 0.73 μmol/L (315 ng/mL). In this study, when CFVs were inhibited, the plasma concentration of OPC-29030 after treatment was begun was 212 ± 30 and 286 ± 48 ng/mL at 20 and 40 minutes, respectively. Thus, the effective plasma OPC-29030 levels in vivo were close to the value of IC₅₀ in the ex vivo experiment. The cessation of OPC-29030 treatment decreased the plasma OPC-29030 levels to <70 ng/mL and restored CFVs in association with significant increases in plasma and intraplatelet 12-HETE levels. Taken together, our data clearly suggest that platelet-derived 12-HETE plays an important role in the thrombotic process of CFVs in this model.

Figure 7. Relationship between platelet aggregation and 12-HETE production stimulated by ADP (A) and U46619 (B). Platelet aggregations were significantly correlated with platelet 12-HETE production (r = 0.82 for ADP and r = 0.86 for U46619).

Figure 8. Intraplatelet cAMP levels before (baseline) and after (initial CFVs) development of CFVs, during OPC-29030 treatment, and after cessation of treatment. Intraplatelet cAMP levels did not change at any given time point.
We used an in vivo model of coronary arterial thrombosis in this study. Although OPC-29030 treatments significantly reduced CFVs in association with decreases in intraplatelet 12-HETE levels, it is not known whether OPC-29030 treatment inhibits platelet aggregation in association with inhibition of platelet 12-HETE production. To address this issue, we investigated the effects of OPC-29030 on ex vivo platelet aggregation and 12-HETE production. ADP and U46619, a thromboxane mimetic, were chosen as platelet agonists in this study because ADP and TXA2 are important mediators of CFVs. In the present study, ADP and U46619 induced platelet aggregation (Figure 5) and 12-HETE production (Figure 6) in a concentration-dependent manner. Both ex vivo platelet aggregation (Figure 5B) and 12-HETE production (Figure 6) were significantly suppressed after OPC-29030 treatments, whereas the vehicle did not have this effect (Figure 5A). Importantly, there was a close linear relationship between platelet aggregation and intraplatelet 12-HETE production for ADP and U46619 (Figure 8). These findings further support the previous studies demonstrating that 12-lipoxygenase inhibitors such as baicalein inhibit in vitro human platelet aggregation induced by the same agonists as used in this study. Thus, our data suggest that OPC-29030 inhibits platelet aggregation through the inhibition of platelet 12-HETE production via the lipoxygenase pathway. Moreover, our data provide evidence that, in the cascade of arachidonic acid metabolism in activated platelets, the lipoxygenase pathway plays an important role in mediating platelet aggregation in addition to the cyclooxygenase pathway, which is implicated in platelet aggregation in the Folts dog model.

OPC-29030 is a novel specific inhibitor of 12-HETE synthesis in activated platelets and can be administered in vivo. Indeed, this agent does not inhibit purified cyclooxygenase activity and does not affect platelet TXA2 production stimulated with thrombin (H. Ito, MS, and coworkers, unpublished work). In the present study, OPC-29030 did not have any effects on the plasma and intraplatelet TXB2 levels in vivo. Thus, OPC-29030 does suppress the 12-HETE synthesis in platelets without a significant effect on the cyclooxygenase pathway. OPC-29030 is a derivative of cilostazol, which is known to inhibit platelet aggregation by elevating intraplatelet cAMP levels. In this study, OPC-29030 at 10 μg · kg⁻¹ · min⁻¹ did not affect intraplatelet cAMP levels. Therefore, the beneficial effects of OPC-29030 on CFVs are unlikely to be mediated via the increases in intraplatelet cAMP levels but are more likely to be mediated via the specific inhibition of 12-HETE synthesis in platelets. In this study, OPC-29030 did not increase plasma and intraplatelet TXB2. The exact mechanisms are unclear. However, previous studies have demonstrated that the inhibition of platelet 12-HETE production prevents the increase in intraplatelet [Ca²⁺], which would have nonspecifically inhibited the activation of phospholipase A2 in platelets, leading to no significant changes in plasma and intraplatelet TXB2 levels in this study. Furthermore, it is possible that 12-HPETE, the precursor of 12-HETE, was a platelet-active lipoxygenase metabolite. However, it has been shown that 12-HPETE inhibits platelet activation and 12-HETE potentiates platelet activation. Hence, this possibility is unlikely.

There are several possible mechanisms by which 12-HETE causes platelet aggregation. First, 12-HETE may potentiate platelet GP IIb/IIIa activation, which is the final common pathway of platelet aggregation and a key factor for coronary thrombosis. Once a platelet is activated, GP IIb/IIIa takes on a new 3-dimensional conformational state and reveals binding sites for fibrinogen. In this work, we studied the effects of inhibition of platelet 12-HETE synthesis on activation of human platelet GP IIb/IIIa because there is no available antibody that reacts with canine platelets. Consequently, OPC-29030 as well as baicalein significantly inhibited human platelet GP IIb/IIIa activation induced by ADP, U46619, and thrombin (Figure 9). The concentration of OPC-29030 used in this in vitro study was comparable to that achieved in a canine model of coronary arterial thrombosis in vivo. Thus, the present findings suggest that platelet 12-HETE causes platelet aggregation via GP IIb/IIIa activation. Second, 12-HETE may upregulate platelet P-selectin, which potentiates the thrombotic process. Recently, we and others have shown that P-selectin plays an important role in
mediating CFVs in the present model. Because P-selectin is an important adhesion molecule for the interaction of platelets and endothelial cells with leukocytes and because it has been demonstrated that OPC-29030 significantly decreases platelet adhesion onto the denuded surface of rat aorta under in vivo dynamic flow conditions (T. Igawa, PhD, and coworkers, unpublished work), the antithrombotic effect of OPC-29030 in this study may be due to the inhibition of platelet adhesiveness through the downregulation of P-selectin.

In conclusion, the present study first demonstrates that OPC-29030, a novel specific inhibitor of 12-HETE synthesis, inhibits platelet aggregation and abolishes CFVs in vivo in association with decreases in both plasma and intraplatelet concentrations of 12-HETE. Furthermore, OPC-29030 inhibited human platelet GP IIb/IIIa activation. Our results strongly suggest that platelet-derived 12-HETE contributes to the pathophysiology of thrombus formation in acute coronary syndromes. Thus, the inhibition of 12-HETE via the lipoygenase pathway in activated platelets could become an attractive therapeutic modality of these syndromes in humans.

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