Most treatment strategies for acute coronary syndromes or adjunctive therapy for PCI are aimed at inhibiting thrombin and platelet activity. The synthetic, direct thrombin inhibitor bivalirudin (Angiomax) is approved for use as an antithrombotic agent in patients undergoing PCI. Bivalirudin binds reversibly to thrombin at both its active site and exosite I, and inhibits both circulating and thrombus-bound thrombin. The safety and efficacy of bivalirudin in patients undergoing elective PCI. Mean plasma levels of bivalirudin were $2.7 \pm 0.5 \mu\text{mol/L}$ during PCI, which correlated with marked inhibition of thrombin-induced platelet aggregation and significantly inhibited cleavage of PAR1. Unexpectedly, bivalirudin also significantly inhibited collagen-platelet aggregation during PCI. Collagen induced a conversion of the platelet surface to a procoagulant state in a thrombin-dependent manner that was blocked by bivalirudin. Consistent with this result, bivalirudin reduced systemic thrombin levels by $>50\%$ during PCI. Termination of the bivalirudin infusion resulted in rapid clearance of the drug with a half-life of 29.3 minutes.

**Conclusions**—Bivalirudin effectively suppresses thrombin-dependent platelet activation via inhibition of PAR1 cleavage and inhibits collagen-induced platelet procoagulant activity as well as systemic thrombin levels in patients undergoing PCI. (Circ Cardiovasc Inter. 2011;4:171-179.)

**Key Words:** thrombin ■ PAR1 PAR4 ■ collagen platelets arterial thrombosis

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**Clinical Perspective on p 179**

Thrombin, a primary mediator of platelet activation and aggregation through the PAR1 and PAR4 thrombin receptors, has been shown to be elevated as a consequence of PCI wherein local thrombin activity in the culprit artery is greatly increased. Whether bivalirudin affects PAR-dependent platelet function and ongoing thrombin generation in patients undergoing PCI is not clear. Moreover, the association between the currently used dosage of bivalirudin and suppression of platelet aggregation by the two major agonists thrombin and collagen has not been determined in patients undergoing PCI.

In the present study, we assessed the degree of protection from thrombin-induced platelet activation conferred by the clinically used dosages of bivalirudin in patients undergoing PCI. We also studied the effect of bivalirudin on systemic thrombin-antithrombin III levels and its effect on collagen-induced platelet aggregation and platelet procoagulant activ-
infarction; CK-MB, creatine kinase MB; PCI, percutaneous coronary intervention.

Patients receiving coronary stents 86%

Known hypersensitivity to bivalirudin, clopidogrel or aspirin, thrombocytopenia

Review Board. Exclusion criteria included current therapy with a 18-gauge needle and a 20-mL syringe prefilled with 2 mL of 4% sodium citrate. Whole blood was transferred into 15-mL polypropylene tubes with EDTA added for a final concentration of 0.25 mmol/L. To prevent loss or dilution of bivalirudin from the patient samples, platelet-rich plasma (PRP) rather than washed platelets was used in all the aggregation experiments. PRP was harvested by centrifuging blood at 700g for 20 minutes at 30°C. To prevent fibrin formation on addition of thrombin agonist, the peptide glycine-L-prolyl-L-arginyl-L-proline (GPRP) (Sigma) was added (1 mmol/L final concentration) to the PRP before performing platelet aggregation. Platelet aggregation was induced by 3 mmol/L to 1 μmol/L thrombin (Hematologic Technologies), 5 μg/mL fibrillar type I collagen (Chronolog), 5 μmol/L SFLLRN or 160 μmol/L AYPGKF (synthesized with C-terminal amides at the Tufts Peptide Core Facility). Platelet aggregation was measured with a Chronolog 560/VS/490 to 2D aggregometer with platelet-poor plasma serving as a blank. Samples were recalculated with CaCl2 (2.5 mmol/L final concentration) before addition of agonists. All reactions were conducted in final volumes of 250 μL at 37°C while stirring at 900 rpm.

**Quantification of Bivalirudin Concentration in Plasma**

Bivalirudin levels in plasma were obtained from PCI patients just before bivalirudin infusion (baseline), immediately following bolus infusion, 30 minutes following the institution of the continuous infusion, or at the end of PCI if the procedure was completed earlier (30 minutes), 5 minutes after termination of infusion (5 minutes POST), 15 minutes after termination of infusion (15 minutes POST), and 2 hours after termination of infusion (2 hours POST). Whole blood was drawn into 6-mL EDTA-containing test tubes and immediately transferred to ice. Platelet-poor plasma samples were stored at −80°C. Platelet-poor plasma samples were shipped on dry ice to Frontage Laboratories (Malvern, PA) for determination of plasma drug levels using a Q-Trap 5000 MS/MS system equipped with an high-performance liquid chromatography tandem mass spectrometry.

**Platelet Aggregation of Blood Samples From Healthy Volunteers**

Blood was obtained from adult healthy volunteers (n=10, 5 males, 5 females) from the greater Boston area recruited by Tufts institutional review board-approved procedures. PRP was obtained from the healthy volunteers in an identical manner as the PCI patients. To obtain the standard bivalirudin inhibition curves, various concentrations (0.1 to 10 μmol/L) of bivalirudin were preincubated with PRP for 2 minutes before addition of thrombin. Platelet aggregation assay was performed in an identical manner as with the PRP from patients. Threshold median effective concentration (EC50) values were plotted as a function of bivalirudin concentration. To measure the effects of the presence of plasma proteins on collagen- and thrombin-induced aggregation, gel-filtered platelets were further prepared from healthy-volunteer PRP using Sepharose 2B columns in modified PIPES buffer as previously described.16 Platelet aggregation was measured using PIPES buffer as control. Inhibitors were added 2 minutes before addition of collagen or thrombin agonists.

**Prothrombinase, Thrombin-Antithrombin III, and Thromboxane B2 Assays**

Thrombin generation was quantified in citrate-anticoagulated human PRP as described16 with simple modifications. In brief, 250 μL of

<table>
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Data are reported as mean±SD. TIMI indicates thrombolysis in myocardial infarction; CK-MB, creatine kinase MB; PCI, percutaneous coronary intervention.
Kimmelstiel et al Platelet Inhibition by Bivalirudin During PCI 173

PRP from healthy donors was recalcified with CaCl₂ (final, 2.5 mmol/L) and stimulated with 5 µg/mL collagen for 5 minutes at 37°C while stirring at 200 rpm. Bivalirudin was added 3 minutes before collagen. Thrombin generation was quantified by addition of 20 mmol/L EDTA in HEPES buffer, pH 7.5, on ice. Thrombin activity was measured using the chromogenic substrate S2238 as described. Quantification of thrombin-antithrombin III (TAT) complexes and Thromboxane B₂ (TXB₂) in the plasma from PCI patients obtained at baseline, 30 minutes after PCI, and 120 minutes after termination of bivalirudin infusion was performed by ELISA using commercially available kits (Affinity Biologicals, Cayman Chemicals, respectively) and following the manufacturer’s protocols. TAT and TXB₂, ELISAs used 20 µL of plasma and 80 µL of buffer per assay well.

Annexin V and Span-12 Binding
Whole blood from healthy donors was diluted 1:10 in modified HEPES-Tyrodes buffer (10 mmol/L HEPES, 137 mmol/L NaCl, 2.8 mmol/L KCl, 1 mmol/L MgCl₂, 12 mmol/L NaHCO₃, 0.4 mmol/L Na₂PO₄, 5.5 mmol/L glucose, and 0.35% BSA, pH 7.4). GPⅡb/Ⅲa at a final concentration of 1 mmol/L was added to prevent fibrin polymerization before addition of agonists. Diluted whole blood was incubated for 10 minutes with bivalirudin or PIPES buffer and then incubated for an additional 10 minutes with 5 µg/mL collagen, 20 to 50 nmol/L thrombin, or both at 37°C. The samples were labeled with CD41a-PE antibody alone or with a mixture of CD41a-PE antibody and annexin V-FITC (BD Biosciences) in the dark at room temperature for 30 minutes. Paraformaldehyde 1% was used to fix the platelets. Analysis was performed on a BD FACSCanto II Flow Cytometry System. Platelets were gated against the CD41a-PE-Ab channel and their characteristic log orthogonal and forward light scatter used to eliminate non–platelet-derived events and debris. For Span-12 antibody binding, PRP anticoagulated with citrate plus 1 mmol/L GPRP was obtained from PCI patients at baseline or after 30 minutes of bivalirudin infusion, and were stimulated with 50 nmol/L thrombin, 5 µmol/L SFLLRN, 160 µmol/L AYPGKF, and 5 µg/mL collagen. Platelet aggregation was performed at 37°C in the presence of 1 mmol/L GPRP. TAT and TXB₂ levels in plasma were analyzed by ELISA as described in Methods. Data are reported as mean±SD, and P values were determined by paired t tests.

Data Analysis
We quantified the degree of platelet inhibition conferred by bivalirudin in PCI patients by using thrombin, AYPGKF, SFLLRN, and collagen as agonists. From our previous bivalirudin spiking study with healthy subjects using a standard deviation (SD) of 10%, and choosing a power of 95% and with an alpha of 0.05, we calculated that we would require at least 9 subjects to obtain a significant result if the true differences between baseline and the bivalirudin treatment groups were as small as 20%. Statistical analyses were performed with GraphPad Prism 5.0A using a paired 2-tailed Student t test. Pairwise comparisons of aggregation results for each agonist were made between baseline (before receiving bivalirudin) and at the 30-minute time point.

Results
Bivalirudin Efficiently Inhibits Thrombin-Induced Aggregation in Platelets from Patients Undergoing PCI
The demographic, clinical, and procedural characteristics of the 22 patients comprising the study population are listed in Table 1. Although this mechanistic study was neither designed nor powered to assess clinical outcomes, no patient experienced an ischemic end point or major or minor bleeding according to thrombolyis in myocardial infarction criteria. We first assessed the effects of bivalirudin on thrombin-, SFLLRN-, and AYPGKF-induced aggregation in platelet-rich plasma from the patients undergoing PCI. Thrombin is a potent agonist of both the PAR1 and PAR4 thrombin receptors. SFLLRN specifically activates PAR1, and AYPGKF specifically activates PAR4. Bivalirudin completely blocked platelet aggregation in response to high concentrations of exogenously added thrombin at the 30-minute time point during the PCI procedure in all patients (Table 2). We next determined the concentration of thrombin that would overcome the inhibitory effects of the infused bivalirudin on platelets from each patient. Bivalirudin markedly right-shifted the thrombin aggregation curves by 26-fold to a mean thrombin EC₅₀ of 256±87 nmol/L in the patients undergoing PCI (Figure 1A through 1C). However, the bivalirudin infusion had no significant effect on mean platelet aggregation in response to the synthetic peptide agonists SFLLRN or AYPGKF in the patients compared with baseline (Table 2).

PAR1 is the high-affinity thrombin receptor on platelets and its activation is highly dependent on interactions between thrombin exosite I and the hirudin-like domain on the PAR1 extracellular domain. Because bivalirudin also binds with high affinity to exosite I of thrombin, we postulated that bivalirudin might inhibit thrombin cleavage of PAR1 on the surface of platelets. The Span12 antibody, which binds only to uncleaved PAR1, was used to quantify thrombin cleavage of PAR1 on the platelet surface. We found that 20 nmol/L thrombin caused 81% loss of Span12 binding to the platelet surface at baseline which was significantly protected by the 30 minutes bivalirudin infusion (Figure 1D and 1E). Similar protection against thrombin cleavage of PAR1 was observed by adding exogenous bivalirudin to platelets from healthy volunteers (Figure 1E).

Pharmacokinetics of Bivalirudin in Patients Undergoing PCI
A previous pharmacokinetic study in PCI patients using the first Food and Drug Administration-approved dosage regimen of bivalirudin based on the Bivalirudin Angioplasty Trial (bolus of 1.0 mg/kg followed by an infusion of 2.5 mg · kg⁻¹ · h⁻¹).
Bivalirudin protects percutaneous coronary intervention (PCI) patients against thrombin-induced platelet aggregation and PAR1 cleavage. (A) Platelets in PRP were isolated from PCI patients at baseline (●) and after 30 minutes of bivalirudin infusion (○), and were stimulated with various concentrations of thrombin. Platelet aggregometry was performed at 37°C in the presence of 1 mmol/L GPRP. Aggregation data from 3 representative patients are plotted as a function of thrombin concentration. (B) The EC50 values of thrombin aggregation for all 22 PCI patients are reported as the median within the first and third quartiles (box). (C) The EC50 of thrombin-dependent aggregation for each patient sample at 30 minutes (●) was plotted as a function of plasma bivalirudin concentration. Overlaid on these patient data are the thrombin EC50 values (mean ± SD) of PRP from healthy individuals (□) plotted as a function of spiked bivalirudin concentration. The solid line represents the least-squares fit of the data and the dashed lines are the mean thrombin EC50 (256 nmol/L) of the PCI patient population and predicted mean bivalirudin concentration (3.1 μmol/L) based on the inhibitory data from the healthy individuals. (D and E) Platelets in PRP was isolated from patients (n = 5) at baseline or 30 minutes PCI, or from healthy donors (n = 3) and challenged with 20 nmol/L thrombin. Bivalirudin (2.7 μmol/L) was spiked into the healthy donor PRP. Surface expression of the intact thrombin cleavage epitope of PAR1 on the platelet surface was then assessed by FACS. FACS results are shown for one representative PCI patient in D and the mean ± SE are shown in E for all the PCI patients and healthy donors, *P < 0.015 by paired-sample t test.

h⁻¹),²¹ yielded mean steady-state bivalirudin plasma levels of 5.6 μmol/L (12.3 ± 1.7 μg/mL) and a terminal half-life of 25 minutes. However, the plasma levels and half-life of bivalirudin have not been determined using the current Food and Drug Administration-approved dosage regimen of bivalirudin established by the REPLACE-2 study, which uses a substantially reduced bivalirudin exposure (0.75 mg/kg bolus and 1.75 mg·kg⁻¹·h⁻¹ infusion) for the duration of the PCI procedure. Therefore, we measured circulating bivalirudin drug levels from 22 PCI patients both during and after termination of bivalirudin infusion. Peak drug levels reached 3.2 ± 0.7 μmol/L at the 5- to 10-minute time point just after the bolus injection (Figure 2, online-only Data Supplement Table). The mean steady-state bivalirudin concentration during PCI was 2.7 ± 0.5 μmol/L, a >50% reduction in the bivalirudin levels relative to patients that received the original 1.0 mg/kg bolus and 2.5 mg·kg⁻¹·h⁻¹ infusion dosage.²²

The distribution of steady-state drug levels in individual patients ranged from a low of 1.7 μmol/L to a high of 3.7 μmol/L. Despite these individual differences, all patients had dramatic right-shifts in their thrombin EC50 curves from a low of 150 nmol/L to a high of 570 nmol/L (Figure 1C). The thrombin-platelet aggregometry dose–response curve determined for a range of bivalirudin concentrations in whole blood gave a predicted mean plasma bivalirudin level of 3.1 μmol/L, which deviated by only 13% from the actual measured mean steady-state value of 2.7 μmol/L in the patient samples (Figure 1C).

After discontinuation of the infusion, bivalirudin was rapidly cleared from the plasma with a mean half-life of 29.3 ± 15.3 minutes (online-only Data Supplement Table). Two hours after discontinuation of the bivalirudin infusion,
residual plasma drug levels (0.2 μmol/L) were only 11% of the steady-state levels.

**Bivalirudin Significantly Decreases Systemic TAT Levels in Patients Undergoing PCI**

Patients with acute coronary syndromes have high systemic levels of various hemostatic markers that indicate an active prothrombotic and procoagulant state that can contribute to arterial thrombosis. The observation that bivalirudin decreases platelet activity leads to the prediction that systemic thrombin levels may be decreased from baseline following infusion of bivalirudin in the patients undergoing PCI. TAT complexes are rapidly formed when antithrombin III irreversibly binds to active thrombin; thus, circulating TAT complexes are a measure of systemic thrombin levels. Indeed, we found that the baseline mean systemic levels of TAT were 215 ± 104 pmol/L (normal range, 10 to 30 pmol/L) in our PCI patients. Given that TAT complexes have a very short plasma half-life of 15 minutes, these highly elevated TAT levels suggest ongoing systemic thrombin generation. To assess the effect of bivalirudin on systemic thrombin, we compared plasma TAT levels at baseline, after 30 minutes of bivalirudin infusion, and 2 hours after cessation of bivalirudin. As shown in Figure 3, the PCI patients had a significant drop in mean systemic TAT levels to 98 ± 41 pmol/L (Table 2), 30 minutes after initiation of the bivalirudin infusion. Notably, there was no significant rebound in mean systemic TAT levels 2 hours after termination of the bivalirudin infusion (Figure 3).

**Bivalirudin Inhibits Collagen-Induced Platelet Activation**

Bivalirudin unexpectedly caused a statistically significant inhibition of collagen-induced aggregation of platelet-rich plasma obtained from the PCI patients at the 30-minute time point compared with baseline (Table 2, Figure 4A). The inhibitory effect of spiked bivalirudin on collagen-induced aggregation in platelet-rich plasma (Figure 4B) was not observed when plasma proteins were removed from the platelet preparations by gel filtration (Figure 4C), consistent with previous data. Addition of exogenous thrombin to plasma-depleted platelets gave 80% platelet aggregation, which was completely blocked by bivalirudin or the direct thrombin inhibitor, PPACK (Figure 4D). To rule out that the observed differences in collagen aggregation were because of variable inhibition of thromboxane pathways, we confirmed that plasma TXB2 levels were unchanged from baseline versus the 30-minute time point in the PCI patients (Table 2). This was expected because the patients were documented long-term aspirin users with 20 of the 22 patients receiving their last aspirin dose 4 hours before the procedure and 2 patients receiving their last aspirin dose just before the procedure.

**Bivalirudin Inhibits Collagen-Dependent Platelet Procoagulant Activity**

Given that inhibition of collagen-induced platelet aggregation by bivalirudin required the presence of plasma proteins (eg,
prothrombin and other coagulation factors), we tested the hypothesis that bivalirudin inhibits collagen-initiated aggregation by suppressing platelet-dependent thrombin generation. We used annexin V binding to assess the appearance of negatively charged phospholipids in individual platelets from PCI patients and healthy donors as a marker of procoagulant activity within the platelet population. Addition of collagen to whole blood obtained from PCI patients at baseline or from healthy volunteers gave a large 6- to 20-fold increase in annexin V binding (30% positive platelets). The collagen-induced increase in annexin V binding was significantly inhibited in the platelets obtained from the PCI patients after 30 minutes of bivalirudin infusion or by spiking whole blood from healthy volunteers (Figure 5A and 5B).

Consistent with previous data, thrombin gave a 2-fold increase in annexin V-positive platelets, which was inhibited in the whole blood obtained from PCI patients after 30 minutes of bivalirudin infusion or by addition of bivalirudin to whole blood from healthy volunteers. The combination of collagen plus thrombin gave a synergistic enhancement in annexin V binding, which was inhibited by bivalirudin in the PCI patients and by exogenously added bivalirudin in the healthy volunteers. Likewise, the direct thrombin inhibitor, PPACK, completely blocked annexin V staining in response to collagen or thrombin (Figure 5B).

Platelets in platelet-rich plasma from healthy volunteers were then stimulated with collagen, and thrombin generation directly measured. As shown in Figure 5C, bivalirudin significantly inhibited thrombin generation triggered by exposure of the platelets to collagen. Together, these data indicate that bivalirudin can suppress collagen-induced platelet aggregation by blocking thrombin-dependent propagation of procoagulant activity on the platelet surface.

Discussion

In the present study, we demonstrated that bivalirudin, at the dose currently used during PCI, provides extensive protection from thrombin-induced platelet aggregation. At this dose, bivalirudin also suppressed systemic thrombin-antithrombin III levels by more than half and significantly inhibited

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**Figure 4. Bivalirudin suppresses collagen-induced platelet aggregation and procoagulant activity.** (A) Platelets in platelet-rich plasma (PRP) from the percutaneous intervention patients at baseline and after 30 minutes of bivalirudin infusion, were stimulated with 5 µg/mL collagen and aggregometry performed. (B) The indicated concentrations (µmol/L) of bivalirudin were spiked into patient PRP (n=3) isolated at baseline and aggregation in response to 5 µg/mL collagen assessed. Identical results were seen with PRP from healthy individuals (data not shown). (C and D) Gel-filtered platelets (healthy volunteers, n=3) were preincubated for 2 minutes with bivalirudin (5 µmol/L) and/or PPACK (100 µmol/L) and then challenged with 5 µg/mL collagen or 2 nM/L thrombin as indicated. **P<0.001 by paired-sample t test with Bonferroni correction.
collagen-induced platelet aggregation and generation of platelet thrombogenic surfaces. These findings demonstrate that bivalirudin has a hitherto unappreciated ability to inhibit both protease-activated receptor and collagen-dependent aggregation and platelet procoagulant activity in patients undergoing PCI. We also provide the first direct evidence that bivalirudin protects against thrombin cleavage of PAR1 on the platelet surface in PCI patients.

Bivalirudin has become a widely used adjunctive therapy in a broad spectrum of patients with coronary artery disease undergoing PCI. Large clinical trials have documented the efficacy of bivalirudin compared with heparin plus GPIIb/IIIa inhibitors.4,5 Our study provides a mechanistic framework to suggest efficacy of bivalirudin as it relates to platelet-dependent ischemic events.

Thrombin is the most potent stimulator of platelet activation and thrombosis.7,27 Thrombin triggers platelet aggregation through the dual actions of both the PAR1 and PAR4 receptors.7 PAR1 is a high-affinity receptor for thrombin by virtue of a hirudin (Hir)-like sequence that resides in its N-terminal extracellular domain.19,28,29 The Hir sequence allows PAR1 to compete with the much more abundant fibrinogen, and as a result, PAR1 is activated by thrombin at subnanomolar concentrations. The present study showed that bivalirudin effectively suppressed cleavage of the extracellular domain of PAR1 on the platelet surface by even supraphysiologic concentrations of 50 nmol/L thrombin.

After cleaving PAR1, thrombin may remain tethered to PAR1 through the Hir sequence where it can cleave nearby

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**Figure 5.** Bivalirudin inhibits collagen-induced platelet procoagulant activity and thrombin generation. (A) Whole blood from percutaneous intervention (PCI) patients (n=5) at baseline or 30 minutes after initiation of PCI was diluted 10-fold into HEPES buffer and was incubated with the indicated agonists (5 µg/mL collagen, 20 nmol/L thrombin, or 5 µg/mL collagen plus 20 nmol/L thrombin). After 15 minutes, the platelet samples were stained with an annexin V antibody, and the percentage of platelets staining (M1 region) with the anionic phospholipid marker was quantified as described in Methods. (B) Whole blood from healthy donors were diluted as the patient samples in A and preincubated with the indicated concentrations of bivalirudin (µmol/L) or 100 µmol/L PPACK before addition of collagen and/or thrombin agonists. The percentage of annexin V binding positive (M1) for both healthy donors (n=6) and patients (n=5) are shown (mean±SD). (C) Platelet-rich plasma from a healthy donor was preincubated in the presence or absence of 5 µmol/L bivalirudin for 2 minutes before the addition of 5 µg/mL collagen. The amounts of thrombin generated (mean±SD of triplicate samples) were determined as described in Methods. The experiment was done with 2 other healthy donors and gave similar results. *P<0.025, #P<0.017, ###P<0.001 by paired-sample t test with Bonferroni correction and *P<0.05 by paired-sample t test.
PAR4, which exists as a heterodimer in association with PAR1.\textsuperscript{8,19} PAR4 is cleaved more slowly than PAR1 mainly because it lacks a functional Hir sequence\textsuperscript{30} and relies on PAR1 to play this critical helper function.\textsuperscript{8} Thus, the Hir analog bivalirudin is an effective inhibitor of the interactions of thrombin with both PAR1 and PAR4. Given that the steady-state levels of bivalirudin achieved during PCI achieved complete blockade of 50 nmol/L thrombin-induced platelet activation, this study would strongly suggest that bivalirudin suppresses both PAR1- and PAR4-dependent platelet activation during PCI. The described effects of bivalirudin-mediated inhibition of thrombin-induced platelet aggregation with an absence of an effect on SFLLRN or AYPGKF indicates that the inhibitory effects of bivalirudin depends on its ability to prevent upstream thrombin activation of the platelet thrombin receptors but, as expected, does not affect platelet aggregation triggered by either of the synthetic PAR1 or PAR4 agonist peptides that bypass the requirement for thrombin cleavage.\textsuperscript{19}

The present study determined that the mean half-life of bivalirudin in the PCI patients was 29 minutes, which is approximately one-third the half-life of unfractionated heparin and is significantly less than the biological half-lives of the 3 commonly used GPIIb/IIIa inhibitors.\textsuperscript{31} This rapid disappearance of bivalirudin from the plasma likely contributes to the relatively low incidence of acute bleeding complications observed in PCI patients that receive bivalirudin monotherapy compared with patients who receive the longer-lived heparin and GPIIb/IIIa inhibitors.

We also provided evidence that bivalirudin negatively regulates on-going thrombin generation in PCI patients. Systemic thrombin, as reflected by antithrombin III-thrombin levels was significantly suppressed by bivalirudin in nearly all patients during the PCI procedure and did not rebound above baseline levels 2 hours after the bivalirudin infusion was terminated. Moreover, the majority of patients had postprocedural TAT levels that remained significantly suppressed at 2 hours compared with baseline. These data support the notion that bivalirudin targets both existing circulating thrombin and interrupts on-going procoagulant activity.

In this regard, we found that bivalirudin suppressed thrombin generation by blocking collagen-initiated platelet procoagulant activity. GPVI is the major collagen receptor that is a highly efficient activator of platelet procoagulant activity.\textsuperscript{32} Indeed, previous studies showed that exposure of platelets to collagen lead to the appearance of a high percentage of negatively charged phospholipids, as reflected by annexin V binding to the platelet surface.\textsuperscript{33,34} The negatively charged phospholipids serve as a critical binding site for factor Va/Xa and accelerate the prothrombinase activity. The thrombin that is generated on the platelet surface, in turn, further activates platelets through the PARs\textsuperscript{26,35,36} and generates more prothrombinase activity by additional thrombin cleavage and activation of factor V. Bivalirudin attenuates this collagen-initiated platelet activation by inhibiting the thrombin-positive feedback loop. However, it remains to be determined whether the observed 11% drop in mean collagen-induced platelet aggregation provides a clinically significant protective effect. Furthermore, it is unknown whether addition of a P2Y12 antagonist such as clopidogrel may either mask or augment these bivalirudin-mediated inhibitory effects on collagen in PCI patients.

Conflicting results have been reported on the efficacy of bivalirudin versus heparin on platelet function using FACS analysis. A recent study\textsuperscript{11} reported that heparin, but not bivalirudin, inhibited subnanomolar thrombin cleavage and internalization of PAR1 on platelets from PCI patients. However, consistent with our earlier work,\textsuperscript{8} a FACS study\textsuperscript{12} showed that spiked bivalirudin blocked thrombin activation of the GPIIb/IIIa receptor and surface expression of P-selectin to a much greater extent than spiking blood with heparin or heparin plus epifibatide. Likewise, another report\textsuperscript{13} confirmed that bivalirudin completely blocks 10 nmol/L thrombin activation of GPIIb/IIIa and P-selectin immediately after the PCI procedure.

In conclusion, we found that bivalirudin infusion during PCI confers a substantial inhibitory effect on both thrombin- and collagen-mediated platelet activation and reduces plasma thrombin concentrations. These effects, along with the short half-life of bivalirudin, likely contribute to its efficacy and safety when used as an adjunctive agent during PCI.

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**Disclosures**

Dr Kimmelstiel has received lecture fees from Eli Lilly. The study sponsor, The Medicines Company, has a commercial interest in Bivalirudin. The sponsor had no input into the study design or analysis, nor in preparation of the manuscript.

**References**

Adjuvant therapy during percutaneous coronary intervention (PCI) with the direct thrombin inhibitor bivalirudin is an effective antithrombotic agent. Despite widespread use, the antplatelet effects of bivalirudin are not well understood. Thrombin, the most potent activator of platelets and arterial thrombosis, exerts its cellular effects by cleaving the G proteincoupled protease-activated receptors PAR1 and PAR4. We found that bivalirudin completely blocked thrombin-mediated platelet aggregation in patients undergoing PCI by preventing cleavage of the PAR1 receptor. Bivalirudin also significantly inhibited collagen-mediated platelet aggregation while reducing circulating thrombin-ATIII levels by >50%. These data suggest that, during PCI, bivalirudin has a previously underacknowledged ability to inhibit both thrombin- and collagen-mediated platelet aggregation as well as platelet procoagulant activity. Our findings quantify the potent antplatelet effects of bivalirudin in PCI patients and provide a mechanistic framework to explain the clinical efficacy of this drug marketed in large clinical trials.

CLINICAL PERSPECTIVE

Adjuvant therapy during percutaneous coronary intervention (PCI) with the direct thrombin inhibitor bivalirudin is an effective antithrombotic agent. Despite widespread use, the antplatelet effects of bivalirudin are not well understood. Thrombin, the most potent activator of platelets and arterial thrombosis, exerts its cellular effects by cleaving the G-protein coupled protease-activated receptors PAR1 and PAR4. We found that bivalirudin completely blocked thrombin-mediated platelet aggregation in patients undergoing PCI by preventing cleavage of the PAR1 receptor. Bivalirudin also significantly inhibited collagen-mediated platelet aggregation while reducing circulating thrombin-ATIII levels by >50%. These data suggest that, during PCI, bivalirudin has a previously underacknowledged ability to inhibit both thrombin- and collagen-mediated platelet aggregation as well as platelet procoagulant activity. Our findings quantify the potent antplatelet effects of bivalirudin in PCI patients and provide a mechanistic framework to explain the clinical efficacy of this drug marketed in large clinical trials.
Bivalirudin Is a Dual Inhibitor of Thrombin and Collagen-Dependent Platelet Activation in Patients Undergoing Percutaneous Coronary Intervention
Carey Kimmelstiel, Ping Zhang, Navin K. Kapur, Andrew Weintraub, Barath Krishnamurthy, Vilma Castaneda, Lidija Covic and Athan Kuliopulos

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## Supplemental Material

**Supplementary Table. Pharmacokinetics of the 0.75/1.75 Bivalirudin Dosage Regimen in Patients undergoing PCI**

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolus dosage of bivalirudin (mg/kg)</td>
<td>0.75</td>
</tr>
<tr>
<td>Infusion rate of bivalirudin (mg/kg/min)</td>
<td>1.75</td>
</tr>
<tr>
<td>Infusion time of bivalirudin (min)</td>
<td>50 ± 30</td>
</tr>
</tbody>
</table>

Plasma half life of bivalirudin (min) 29.3 ± 15.3

### Plasma bivalirudin (µmol/L)

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Value ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0</td>
</tr>
<tr>
<td>After bolus infusion</td>
<td>3.2 ± 0.7</td>
</tr>
<tr>
<td>30 min or the end of PCI</td>
<td>2.7 ± 0.5</td>
</tr>
<tr>
<td>5 min POST termination</td>
<td>2.4 ± 0.5</td>
</tr>
<tr>
<td>15 min POST termination</td>
<td>1.8 ± 0.6</td>
</tr>
<tr>
<td>120 min POST termination</td>
<td>0.2 ± 0.2</td>
</tr>
</tbody>
</table>

### ACT (s)

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Value ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>After bolus infusion</td>
<td>349 ± 40</td>
</tr>
<tr>
<td>30 min or the end of PCI</td>
<td>333 ± 26</td>
</tr>
</tbody>
</table>

Data are reported as Mean ± SD.