Comparison of PD0348292, a selective factor Xa inhibitor, to antiplatelet agents for the inhibition of arterial thrombosis

Krzysztof Karnicki², Robert J. Leadly Jr.³, Sangita Baxi³, Thomas Peterson³, Waldemar Wysokinski¹,², Robert D. McBane, II,¹

¹Division of Cardiology, ²Section of Hematology Research Mayo Clinic, Rochester, Minnesota, USA; ³Cardiovascular Biology, Pfizer Global Research and Development, Ann Arbor, Michigan, USA

Summary
The objective of this study was to determine if orally-administered PD0348292, a direct specific factor Xa inhibitor, inhibits thrombosis following porcine carotid arterial injury comparably to aspirin or clopidogrel alone or in combination. We further sought to determine whether the antithrombotic efficacy in vivo could be predicted using an ex-vivo perfusion chamber. Oral treatments included: PD0348292 (0.4, 0.9, or 4.3 mg/kg); PD0348292 (0.4 mg/kg) plus aspirin (325 mg); aspirin; clopidogrel (75 mg); aspirin plus clopidogrel; or vehicle (n=6–10/group). Aspirin and clopidogrel were administered 27 and four hours pre-injury and PD0348292 or vehicle was administered four hours pre-injury. Both carotid arteries were crush-injured, and thrombus was measured by detection of ¹¹¹In-platelets over 30 minutes. Prior to injury, the antithrombotic efficacy was assessed by ex-vivo perfusion chamber platelet deposition. PD0348292 produced dose-dependent prothrombin time (0.9- to 2.9-fold) and aPTT (1.4- to 2.5-fold) prolongations. Bleeding times were significantly prolonged in each active drug group compared to vehicle, but were not significantly different between drug groups. PD0348292 significantly inhibited arterial platelet deposition (x10⁶/cm²) at 4.3 (549 ± 1,066), 0.9 (399 ± 162) and 0.4 mg/kg (531 ± 470) compared to vehicle (2,242 ± 1,443). Aspirin (992 ± 973), clopidogrel (537 ± 483), clopidogrel plus aspirin (228 ± 66) or PD0348292 plus aspirin (558 ± 317) also significantly inhibited platelet deposition, although these values were not significantly different than with any dose of PD348292. Perfusion chamber platelet deposition correlated significantly with in-vivo anti-thrombotic response. In conclusion, PD0348292 inhibited arterial thrombosis comparable to aspirin plus clopidogrel. Perfusion chamber methodology may be useful in predicting in-vivo antithrombotic efficacy.

Keywords
Thrombosis, platelets, anticoagulants, factor Xa

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Introduction
Platelets are a key component in the pathophysiology of acute coronary syndromes (1). Current guidelines support the use of aspirin and clopidogrel for secondary prevention (2). These agents, however, target-specific activation pathways leaving the platelet responsive to the most potent physiologic agonist, thrombin. They also have limitations regarding treatment failures (so-called “resistance”) and irreversibility. These limitations have led to continued research to discover antithrombotic agents which mitigate these shortcomings and which will provide broad-based treatment for multiple thrombotic indications (e.g. venous and arterial thrombosis). We have previously shown that specific inhibition of either thrombin (by the direct thrombin inhibitor hirudin) or prothrombinase (by the direct factor [F] Xa inhibitor ZK-807834/C1-1031 or tick anticoagulant peptide [TAP]), when given intravenously, inhibits platelet deposition following acute arterial injury and also results in prompt “de-thrombosis” of newly formed thrombi (3–6) In human studies, several intravenously-administered FXa inhibitors have been shown to inhibit ex-vivo platelet deposition in extracorporeal chambers (7–9). Additionally, both direct thrombin inhibitors and FXa inhibitors provide effective prevention and treatment in humans with venous thromboembolism or atrial fibrillation (10–13).

PD0348292 is an orally-active, potent (FXa Ki=0.32 ± 17 nM), competitive, reversible, and highly selective active-site inhibitor of FXa (14). PD0348292 is >1,000-fold selective for Xa...
over related serine proteases, including thrombin, activated protein C, plasmin, tissue plasminogen activator, and trypsin (14). The objective of this study was to assess the hypothesis that orally-administered PD348292 would effectively inhibit thrombosis following porcine carotid arterial injury comparably to currently available antiplatelet therapy with either aspirin or clopidogrel alone or in combination. An additional treatment group of low-dose PD348292 plus aspirin was included to examine the efficacy of this combined therapy versus either PD-348292 alone or the combination of aspirin and clopidogrel. Although many studies have been performed using ex-vivo perfusion chambers to assess antithrombotic efficacy of novel antithrombotic agents, there is little data available that demonstrates a relationship between ex-vivo perfusion chamber efficacy and in-vivo arterial antithrombotic efficacy. Consequently, we investigated whether the in-vivo antithrombotic response could be predicted using an ex-vivo perfusion chamber assay using several drugs working via different mechanisms.

Materials and methods

Animals

Four-month-old, pre-estrus, female pigs of the Babcock 4-way cross stock (a mixture of Landrace, Yorkshire, Hampshire, and Duroc breeds) were purchased from local vendors through the Mayo Clinic Section of Veterinary Medicine and housed at the Mayo Institute Hills Facility. The study was approved by the Mayo Clinic Animal Care and Use Committee and conformed to the National Institutes of Health and United States Department of Agriculture guidelines.

Induction of thrombosis

Anesthesia of pigs, 111 In-platelet labeling, and carotid arterial crush injuries were performed as described previously (2). Twenty-seven hours (h) prior to the procedure, the pigs received clopidogrel (75 mg; n=6), aspirin (325 mg; n=8), clopidogrel plus aspirin (n=8), or vehicle control (n=10). These agents (or vehicle) were then re-administered 4 h prior to thrombus induction (Fig. 1). Other treatment groups received oral PD0348292 at 0.4, 0.9, or 4.3 mg/kg (n=6) or PD0348292 at 0.4 mg/kg plus aspirin (325 mg; n=6) four hours prior to thrombus induction. The dose of aspirin (Bayer Pharmaceuticals, Wayne, NJ, USA) was chosen based on clinical trial data and to ensure sufficient absorption to promote maximal cyclooxygenase inhibition in our model (16–18). The dose of clopidogrel (Bristol-Myers Squibb, Princeton, NJ, USA) was chosen based on contemporary clinical trial experience in patients with symptomatic carotid disease (19).

At the time of the procedure, both carotid arteries were injured by six serial hemostat crushes of 5 s duration, interspersed with a 3-second (s) rest period, with each subsequent injury visually abutting the prior injury site. The thrombi were then allowed to propagate for 30 minutes (min). At the end of each preparation, injured arterial segments were harvested and assayed for 111 In content in a scintillation counter. For each experiment, the platelet deposition was averaged for both carotid arteries. To objectively assess blood loss associated with each antithrombotic agent, haemoglobin and hematocrit were compared before and after each experiment (Coulter Model ZM; Beckman-Coulter, Fullerton, CA, USA).

Clotting assays

At 27 h and 4 h prior to the procedure and at baseline, 15 and 30 min after thrombus initiation, blood samples were collected into 1:9 volume of 3.2% Na citrate and centrifuged for 20 min at 1,500 g. The plasma samples were then snap-frozen and stored in aliquots at –70°C. Whole blood activated clotting times were measured with the Hemachron ACT device. Prothrombin times and activated partial thromboplastin times were measured with the ACL9000 coagulation analyzer (Beckman Coulter, Fullerton, CA, USA). The prothrombin time reagent, Innovan, and the activated partial thromboplastin time reagent, ActinFS, were obtained from Dade-Behring (Deerfield, IL, USA).

The FXa clotting assay was used to determine the inhibitory effect of PD0348292. Plasma samples were diluted 1:1 with Factor-X-deficient human plasma (Helena Laboratories Corp., Beaumont, TX, USA) and then clotting was initiated with recombinant tissue factor (RecombiPlasTin, Instrumentation Laboratory, Barcelona, Spain). Clotting times were measured with the ACL 9000 coagulation analyzer and compared to untreated pig plasma diluted with varying amounts of FX-deficient human plasma. FXa activity was analyzed by complete activation of the endogenous FX by RVV (DiaPharma, West Chester, PA, USA) and CaCl₂, and subsequent measurement of plasma activity by cleavage of a specific FXa paranitroanilide substrate (Spectrazyme Xa, American Diagnostica, Greenfield, CT, USA). In order to measure the thrombin generated for one hour after in vitro clot formation, total thrombin generation was assessed by utilizing a fluorescent substrate specific for thrombin. Thrombin generation studies were performed as previously described with the modification of 300 mM CaCl₂ (rather than 750 mM CaCl₂) in the activation buffer (20).

Platelet aggregation assay

To assess the response of porcine platelets to each of the antagonists, venous blood was collected by venipuncture using a two-syringe technique and combined with 1:9 volume of 3.8% sodium citrate. Platelet-rich plasma (PRP) was prepared by centrifugation (700 x g for 10 min at room temperature) for platelet aggregometry testing (Chrono-Log Corporation, Havertown, PA, USA). Platelet-poor plasma (PPP) was prepared by re-cen-

Figure 1: Flow diagram of interventions and measurements. PD-0348292 was only administered at “Dose 2”. All other treatments were administered at “Dose 1” and “Dose 2”. Samples for measurement included platelet aggregation (PA), bleeding time (BT), and coagulation assays (Coag).
trifugation of a sample aliquot at 2000 X g for 10 min. The platelet count of PRP was adjusted to 250 X 10^9/l by addition of an appropriate volume of autologous PPP. Aggregations were initiated with either ADP (20 µM) or arachidonate (0.5 mM) and recorded for 5 min. Maximal aggregation was reported as the greatest increase in light transmission within 5 minutes after addition of agonist. Platelet aggregations were performed at baseline, 27 h prior to the procedure, and again immediately prior to surgery. Results are reported as fold change of the maximal aggregation at surgery relative to baseline values.

**Ear bleeding time**
Following sedation, the ear was immersed in warm saline at 37°C for 5 min. Then the ear was supported with cork and two small stab incisions were made at the ear margin using a #11 surgical blade. The incised ear was then placed back into warm saline and carefully observed for time to bleeding cessation. Time to cessation was taken as an average of the two incisions. Ear bleeding times were also performed at baseline, 27 h prior to the procedure, and again immediately prior to surgery. Results are reported as fold change over baseline.

**Perfusion chamber**
Arterial substrate preparation, Badimon flow chamber perfusion, and quantification of autologous 111In-labeled platelet (x10^6/cm²) deposition were performed as previously described (21, 22). Briefly, a porcine aorta was surgically harvested, immersed in 2-methylbutane, frozen in liquid nitrogen and stored at −70°C until use. Longitudinal segments were cut from a single aorta, sandwiched between two sheets of teflon and compressed using an industrial hydraulic platten press (Carver Inc. Wabasha, IN, USA). Compressed arterial segments were placed inside the perfusion chamber and were perfused with porcine whole blood taken directly from a femoral arterial catheter for 5 minutes at a flow rate of 10 ml/min (shear rate of 1,690 1/sec) drawn by a peristaltic pump and then assayed for 111In content. Chambers were run in duplicate just prior to carotid arterial injury.

**Drug concentration**
Arterial blood samples were also collected in heparinized tubes at 27 and 4 h prior to the procedure and at 0, 15, and 30 min after thrombus induction. Samples were centrifuged for 10 min at 3,000 x g at 4°C and stored at −70°C for subsequent analysis. Plasma levels of PD 0348292 were determined by liquid chromatography-mass spectroscopy analysis. The level of detection for this assay is 10 ng/ml.

**Statistics**
All values are presented as mean ± SEM. The unpaired Student’s t-test was used to assess overall treatment effects of continuous normally distributed data between groups. For data that was non-normally distributed, the Wilcoxon signed rank test was used. Paired Student’s t-test was used to compare treatment effects of antithrombotic agents before and after chamber perfusion and to compare haemoglobin, hematocrit and platelet counts before and after each surgery. Linear and non-linear regression analysis was performed using GraphPad Prism. Statistical significance was set at p≤0.05.

**Results**
To assess the efficacy of inhibition of platelet thrombus formation in vivo, a crush injury was performed on both carotid arteries of each animal and the thrombi were allowed to propagate for 30 minutes prior to vessel harvest (Fig. 2A). Administration of PD348292 at 0.4, 0.4 (plus aspirin), 0.9, and 4.3 mg/kg produced dose proportional increases in plasma drug concentration of 56 ± 36, 36 ± 14, 128 ± 19, and 417 ± 81 ng/ml, respectively. PD348292 yielded significant reductions in carotid platelet deposition at 4.3 mg/kg (549 ± 1066 x 10^6/cm²), 0.9 (399 ± 162 x 10^6/cm²) and 0.4 mg/kg (531 ± 470 x 10^6/cm²) compared

Figure 2: Platelet-rich thrombus inhibition. Thrombus inhibition in vivo (Fig. 2A) was quantified by measuring autologous 111In-platelet content in harvested arteries. Compared to vehicle control, platelet deposition was significantly reduced in animals receiving any active drug. Platelet deposition with PD0348292 at any dose was not significantly different than platelet deposition with aspirin or clopidogrel alone, or in combination. Ex-vivo perfusion chamber experiments revealed qualitatively similar inhibition for each agent (Fig. 2B), but platelet deposition was more variable within treatment groups. Symbols represent a significant difference (p < 0.05) compared to vehicle (asterisks) and a significant difference compared to clopidogrel plus aspirin (dagger). Wilcoxon signed rank test was used for non-normally distributed data (Panel A: PD0348292 4.3 mg, aspirin; Panel B: PD0348292 0.9 mg, 0.4 mg plus aspirin, aspirin, clopidogrel).
To determine whether an ex-vivo assay could predict the in-vivo thrombotic response, platelet deposition was assessed using an ex-vivo perfusion chamber assay prior to arterial injury (Fig. 2B). Chamber platelet deposition was significantly reduced for PD0348292 4.3 mg/kg (104 ± 55 x 10⁶/cm²), PD0348292 0.4 mg/kg (181 ± 46 x 10⁶/cm²), aspirin (211 ± 130 x 10⁶/cm²), clopidogrel (139 ± 136 x 10⁶/cm²), and clopidogrel plus aspirin (92 ± 34 x 10⁶/cm²) relative to vehicle control (313 ± 135 x 10⁶/cm²). There was no significant difference in platelet deposition between the PD0348292 4.3 mg/kg, clopidogrel plus aspirin, and the clopidogrel alone groups. Figure 3 illustrates the correlation between ex-vivo and in-vivo antithrombotic efficacy for all treatments evaluated in this study (p<0.0001).

Apparent outliers for in-vivo platelet deposition following carotid injury were carefully examined for possible explanations for the lack of antithrombotic drug effect. One pig in the PD0348292 4.3 mg/kg group presented with diarrhea and had plasma drug concentrations close to the detection level of the assay (<10 ng/ml), so data from this animal was not included in this report. Similarly, one animal in the PD0348292 0.4 mg/kg group had plasma drug concentrations of approximately 10 ng/ml, no changes in coagulation parameters, and in-vivo platelet deposition values of approximately 3500 x 10⁶/cm² (>>3xSD for this treatment group) suggesting that technical difficulties may have been responsible for the low exposure, so data from this animal was also not included in the analysis in Figure 2. The remaining apparent platelet deposition outlier in the PD0348292 4.3 mg/kg group had plasma drug concentrations and coagulation parameter responses comparable to the other animals in the same group, so it was included in the data analysis. In the clopidogrel group, one animal had in-vivo and ex-vivo platelet deposition values that were approximately three-fold over the corresponding group average. This pig also had a platelet aggregation response to ADP that was increased by 29% above the pre-treatment response. Because we didn’t measure the active clopidogrel metabolite in plasma, it was difficult to distinguish whether this animal received and absorbed the drug or was simply a non-responder; therefore this data-point was included. Platelet aggregation responses for the other five animals in the clopidogrel-treated group were inhibited in response to ADP.

To assess the effects of these agents on primary haemostasis, ear bleeding times and platelet aggregation studies were performed. Ear bleeding times were significantly prolonged in each group receiving active drug compared to baseline values or vehicle control (Fig. 4), but were not different between treatment groups.

Porcine platelets responded as anticipated to clopidogrel or aspirin in platelet aggregation studies. Clopidogrel alone, or in combination with aspirin, resulted in significant inhibition of ADP-induced ex-vivo platelet aggregation (42% inhibition). The platelet response to arachidonate was essentially eliminated in animals receiving aspirin alone or in combination with other agents. Platelet aggregation in response to arachidonate was not affected by clopidogrel. PD0348292 alone at all three doses did not significantly change ADP- or arachidonate-induced ex vivo platelet aggregation (Table 1).

Oral administration of PD0348292 dose-dependently prolonged clotting assays and inhibited measures of FXa activity.
(Fig. 5). The mean prothrombin time ratio was 2.9 ± 0.3 with PD0348292 4.3 mg/kg and 1.4 ± 0.1 with 0.9 and 0.4 mg/kg. The mean aPTT fold-change from baseline was 2.5 ± 0.2, 1.5 ± 0.1, and 1.4 ± 0.1 at PD0348292 doses of 4.3, 0.9, and 0.4 mg/kg, respectively. ACT prolongation, also dose dependent, was 2.2-, 1.6-, and 1.3-fold greater than baseline at PD0348292 doses of 4.3, 0.9, 0.4 mg/kg, respectively. Antiplatelet therapy did not significantly alter any of the coagulation parameters. PD0348292 dose-dependently inhibited FXa, as indicated by direct measures of FX activity or inhibition of thrombin generation (Fig. 5C-F). At the highest drug concentration, FXa clotting activity and total thrombin generation were reduced by 83 ±3 % and 78 ±5 %, respectively.

No excessive surgical bleeding was noted irrespective of the antithrombotic agent or dose. The pre- and post-procedural mean hematocrit values did not differ significantly for PD0348292 at 4.3 (27.5 ± 1.9 vs. 27.4 ± 2.4%), 0.9 (28.1 ± 1.3 vs. 27.7 ± 1.9%), 0.4 mg/kg (24.3 ± 8.9 vs. 24.2 ± 8.9%), PD0348292 at 0.4 mg/kg plus aspirin (24.1 ± 7.0 vs. 24.0 ± 7.1%), clopidogrel (26.2 ± 10.9 vs. 26.5 ± 10.3%), aspirin (29.4 ± 2.2 vs. 28.4 ± 2.3%), clopidogrel plus aspirin (29.0 ± 1.6 vs. 29.1 ± 2.5%), or vehicle control (29.1 ± 1.9 vs. 29.3 ± 2.4%). The pre- and post-haemoglobin values likewise did not differ significantly (data not shown).

**Discussion**

Conventional antiplatelet agents may not provide adequate antithrombotic therapy for patients with arterial disease. Aspirin therapy in patients with coronary artery disease is associated with only a 23% reduction in vascular events (23). Consequently, many patients will suffer a thrombotic event despite daily aspirin therapy. One proposed explanation for these clinical “aspirin failures” is that platelets from these individuals are not effectively inhibited by aspirin and therefore they are referred to as “aspirin resistant”. The prevalence of aspirin “resistance” determined by a lack of in-vitro laboratory inhibition varies from 5 – 60% depending on the patient population studied and criteria used for defining the condition (24–28). As we learn more about aspirin “resistance”, the use of clopidogrel, a platelet P2Y12 ADP receptor antagonist, has grown considerably over the past decade. However, as many as 15 – 25% of patients taking clopidogrel may not respond appropriately to this drug (29–31). Indeed, platelets from a minority of patients may actually aggregate more robustly in the presence of clopidogrel (29). When given in the setting of elective or urgent PCI and stenting, clopidogrel non-responders have been shown to experience more frequent arterial thrombotic complications of their procedure including stent thrombosis (30). Due to the limitations and uncertainty regarding conventional antithrombotic therapy, the enthusiasm for discovering and developing improved antithrombotic agents for the treatment and prevention of arterial thrombosis remains strong. This enthusiasm has led to the discovery of orally-active, selective inhibitors of FXa (32). Traditionally, FXa inhibitors are evaluated initially for treatment and prevention of venous thrombosis in order to obtain efficacy and safety data without the worries of concomitant antiplatelet therapy. Subsequently, many of these agents will be evaluated for arterial thrombotic indications, in which anti-platelets agents are considered the standard-of-care.

The current study directly compares the antithrombotic efficacy of a novel, selective, reversible small molecule inhibitor of FXa to conventional antiplatelet therapy with aspirin and clopidogrel in two well-documented models of platelet-dependent arterial thrombosis (in vivo and ex vivo). The findings of this study support the hypothesis that targeting FXa for antithrombotic therapies provides inhibition of arterial thrombosis that is equally effective as conventional antiplatelet therapy with aspirin and clopidogrel. The direct FXa inhibitor, PD0348292, when administered orally, reduced platelet-rich thrombus formation both in vivo and ex vivo. These effects were similar to clopidogrel alone or in combination with aspirin despite the fact

<table>
<thead>
<tr>
<th>Table 1: Platelet aggregation and plasma PD2348292 concentration.</th>
<th>ADP 20 mM (% change from baseline ± SEM)</th>
<th>Arachidonate (% change from baseline ± SEM)</th>
<th>PD0348292 concentration (ng/ml ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control</td>
<td>117 ± 11</td>
<td>163 ± 64</td>
<td>BLQ</td>
</tr>
<tr>
<td>PD0348292 (4.3 mg/kg)</td>
<td>109 ± 6</td>
<td>92 ± 55</td>
<td>417 ± 81</td>
</tr>
<tr>
<td>PD0348292 (0.9 mg/kg)</td>
<td>95 ± 4</td>
<td>115 ± 40</td>
<td>128 ± 19</td>
</tr>
<tr>
<td>PD0348292 (0.4 mg/kg)</td>
<td>126 ± 23</td>
<td>229 ± 127</td>
<td>56 ± 13</td>
</tr>
<tr>
<td>PD0348292 (0.4 mg/kg) plus ASA (325 mg)</td>
<td>109 ± 11</td>
<td>0 ± 0</td>
<td>36 ± 14</td>
</tr>
<tr>
<td>ASA (325 mg)</td>
<td>131 ± 13</td>
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<td>BLQ</td>
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<tr>
<td>Clopidogrel (75 mg)</td>
<td>49 ± 13</td>
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<td>BLQ</td>
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<td>Clopidogrel (75 mg) plus ASA (325 mg)</td>
<td>59 ± 7</td>
<td>0 ± 0</td>
<td>BLQ</td>
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Values measured from samples obtained at 4 h prior to surgery compared to baseline samples obtained 27 h prior to surgery (pre-treatment). Plasma drug concentration was determined from samples obtained at the initiation of thrombus formation (i.e. 5 h after drug administration). *p<0.05 vs. vehicle control; BLQ: below level of quantification (<10 ng/ml).
that these antiplatelet agents were dosed twice over 27 hours compared to PD0348292 which was given just once four hours prior to carotid injury. In addition, markers of bleeding risk and surgical bleeding were comparable between PD0348292 and aspirin plus clopidogrel treated animals.

Similar antithrombotic efficacy was noted across the dose range studied in animals receiving the FXa inhibitor (Fig. 2A). Although there was a dose effect noted with the in-vitro assays, the in-vivo data suggest that both circulating and thrombus bound FXa was adequately inhibited at the lowest dose (Fig. 5). To define the threshold of FXa inhibition as it relates to thrombus deposition in vivo, a smaller dose of PD0348292 is required. At these doses, there was a significant prolongation in the bleeding times (Fig. 4). These data suggest that prothrombinase activity is important in haemostasis, at least in pigs.

These findings indicate that FXa inhibition alone provides effective primary prophylaxis in the setting of a potent stimulus of arterial injury as is seen in these models. Moreover, the addition of aspirin to the lowest dose of PD0348292 did not enhance the activity of PD0348292 alone. Gastrointestinal irritation, aspirin “resistance”, and complication of combination therapy could thus be avoided with the use of a FXa inhibitor alone for arterial

**Figure 5**: Anticoagulant and anti-FXa activity of PD0348292. Plasma samples were taken just prior to arterial injury, assessed for various parameters and plotted versus plasma drug levels. A strong correlation was noted between plasma PD0348292 drug levels and prothrombin time (Fig. 5A; \( r = 0.88 \)), activated partial thromboplastin time (Fig. 5B; \( r = 0.86 \)), activated clotting time (Fig. 5C; \( r = 0.71 \)), FX clotting (Fig. 5D; \( r = 0.78 \)), Russell Viper-Venom (RVV) Test (Fig. 5E; \( r = 0.91 \)), and total thrombin generation (TG: Fig. 5F; \( r = 0.77 \)).
thrombosis. In contrast, when aspirin was combined with clopidogrel, this produced a 4.4-fold reduction in platelet deposition compared to aspirin alone and a non-significant 2.4-fold reduction in platelet deposition compared to clopidogrel alone. These findings are consistent with clinical findings and dosing instructions for clopidogrel in patients with acute coronary syndrome, i.e. aspirin should be administered with clopidogrel (33).

Another potential advantage of FXa inhibitors, such as PD0348292, is the ability to easily monitor their pharmacodynamic activity with routine clinical tests. Figure 5 demonstrates the predictable responses of PD0348292 in a number of routine (PT, aPTT, and ACT), FXa-selective (FX Clotting and RVV), and global (Total Thrombin Generation) tests of coagulation system activity. Some of these tests are currently performed at the bedside and are even available for use in the patient’s home. Although FXa inhibitors may ultimately not require monitoring for routine use, the ability to easily and rapidly monitor their activity will allow urgent care clinicians to make quick decisions regarding treatment and intervention options. Moreover, unlike clopidogrel and aspirin, PD0348292 is a reversible antithrombotic agent (dog $t_{1/2}$ = 4.9 h), which permits timely surgical interventions without the necessary delay or complications required for patients receiving irreversible antiplatelet therapy (14).

This study demonstrates a significant correlation between in-vivo carotid artery platelet deposition and ex-vivo chamber platelet deposition, regardless of the mechanism of action of the agents tested. The drawback of the current experimental design was that dose-response data were not obtained for aspirin and clopidogrel. Nevertheless, the in-vivo and in-vitro measures correlated well over a wide range of antithrombotic efficacy, demonstrating the potential of the ex-vivo chamber for use in demonstrating proof-of-concept for new agents and for making dose projections for later stage clinical studies. The translatability of the ex-vivo chamber results in human subjects to in-vivo human efficacy predictions would be useful for clinical development of novel agents. Specifically, the chamber can be used in such a way that the drug is injected into the flowing blood outside of the systemic circulation so that the subject is not directly exposed to the drug. The plasma concentration of the drug and desired biomarkers can be measured in the effluent blood that has passed over the chamber. These data can then be used to approximate effective doses to be used in patients in later clinical trials, thus increasing confidence in the drug or mechanism and reducing the time and expense involved in large, costly dose-ranging studies.

In conclusion, the oral delivery of the direct FXa inhibitor, PD0348292, inhibits arterial thrombosis with efficacy similar to currently available anti-platelet therapy with aspirin plus clopidogrel. This inhibitor may provide an attractive alternative to traditional anti-platelet or anti-coagulant therapy in the prevention of arterial thrombosis. In addition, ex-vivo perfusion chamber methodology may be a useful tool for predicting the in-vivo efficacy of novel antithrombotic agents, thereby decreasing the cost and time involved in developing these agents.

References


