Evaluation of PAI-039 [[1-Benzyl-5-[4-(trifluoromethoxy)phenyl]-1H-indol-3-yl](oxo)acetic Acid], a Novel Plasminogen Activator Inhibitor-1 Inhibitor, in a Canine Model of Coronary Artery Thrombosis

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ABSTRACT
We tested a novel, orally active inhibitor of plasminogen activator inhibitor-1 (PAI-1) in a canine model of electrolytic injury. Dogs received by oral gavage either vehicle (control) or the PAI-1 inhibitor PAI-039 [[1-benzyl-5-[4-(trifluoromethoxy)phenyl]-1H-indol-3-yl](oxo)acetic acid] (1, 3, and 10 mg/kg) and were subjected to electrolytic injury of the coronary artery. PAI-039 caused prolongation in time to coronary occlusion (control, 31.7 ± 6.3 min; 3 mg/kg PAI-039, 66.0 ± 6.4 min; 10 mg/kg, 56.7 ± 7.4 min; n = 5–6; p < 0.05) and a reduced thrombus weight (control, 7.6 ± 1.4 mg/kg; 3 mg/kg PAI-039, 3.6 ± 1.0 mg; p < 0.05). Although occlusive thrombosis was observed across all groups based upon the absence of measurable blood flow, a high incidence (>60%) of spontaneous reperfusion occurred only in those groups receiving PAI-039. Spontaneous reperfusion in the 10 mg/kg PAI-039 group accounted for total blood flow (area under the curve of coronary blood flow) of 99.6 ± 11.7 ml after initial thrombotic occlusion (p < 0.05 compared with control). Plasma PAI-1 activity was reduced in all drug-treated groups (percentage of reduction in activity p < 0.05; 10 mg/kg PAI-039), whereas ADP-, 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin F2α (U46619)-, and collagen-induced platelet aggregation, as well as template bleeding and prothrombin time, remained unaffected by PAI-039. Ex vivo clot lysis analysis revealed normal clot formation but accelerated clot lysis in PAI-039-treated groups. The pharmacokinetic profile of PAI-039 indicated an oral bioavailability of 43 ± 15.3% and a plasma half-life of 6.2 ± 1.3 h. In conclusion, PAI-039 is an orally active prothrombolytic drug that inhibits PAI-1 and accelerates fibrinolysis while maintaining normal coagulation in a model of coronary occlusion.

Plasminogen activator inhibitor-1 (PAI-1) is the primary inhibitor of tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (uPA), and functionally serves to suppress tissue and plasma fibrinolysis. PAI-1 was initially discovered in 1977 as a “fast-inactivator” of tPA (Wiman and Collen, 1977), and its role in the development of acute disorders such as deep vein thrombosis and myocardial infarction has recently been extended to tissue remodeling, atherosclerosis, and cancer (Stefansson et al., 2003). PAI-1 is a member of the serpin family of proteins (Potempa et al., 1994) and exists in multiple conformations of which a minor component, the “active” form, exhibits inhibitory effects against tPA and uPA (Lawrence, 1997). Inhibitory monoclonal antibodies to PAI-1 have been used in preclinical models of acute thrombosis and shown to enhance endogenous fibrinolysis (Biemond et al., 1995; van Giezen et al., 1997). Due in part to its structural complexity, modulation of PAI-1 by orally active drugs has not been fully realized, despite considerable effort in this area over the past decade (Wu and Zhao, 2002; Gils and Declerck, 2004).

We recently identified a small molecule inhibitor directed specifically against the active, inhibitory conformation of the serpin (Elokdah et al., 2004). This molecule, tiplaxtinin...
(chemical designation PAI-039), performed as an oral profibrinolytic agent in preliminary studies in a rodent model of acute arterial thrombosis. The goal of the present study was to further characterize PAI-039 in a canine model of coronary thrombosis, specifically focusing on endpoints of recanalization and hemostasis. We show that this orally active inhibitor of PAI-1 both stimulates endogenous fibrinolysis and restores coronary blood flow without increasing template bleeding time.

Materials and Methods

The procedures used in all animal studies were conducted in accordance with the guidelines of the Wyeth Collegeville Animal Care and Use Committee and conform to the standards in The Guide for Care and Use of Laboratory Animals (National Institutes of Health no.86-23). PAI-039 ([1-benzyl-5-[4-(trifluoromethoxy)phenyl]-1H-indol-3-yl]oxo)acetic acid] was synthesized by Wyeth Research and formulated in a solution of 2.0% Tween 80/0.5% methylcellulose (Fluka BioChemika, Neu-Ulm, Switzerland) for intragastric dosing. The suspension was sonicated for 1 min followed by stirring for 1 h at room temperature. The structure of PAI-039 was previously published under the name tiplaxtinin (Elokdah et al., 2004). Recombinant human tPA (Activase) was manufactured by Genentech (South San Francisco, CA). All remaining reagents were purchased from Sigma-Aldrich (St. Louis, MO).

In Vivo Dog Coronary Thrombosis Model. Dogs were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and ventilated with room air with the use of a cuffed endotracheal tube and a Harvard respirator (Harvard Apparatus Inc., South Natick, MA) adjusted to deliver a tidal volume of 30 ml/kg (12 respirations/min). The right femoral artery and vein were isolated and cannulated for blood pressure monitoring (Millar Mikro-tip catheter; Millar Instruments Inc., Houston, TX) and blood sampling, respectively. Figure 1 illustrates the experimental protocol in detail. Ninety minutes after intragastric administration of vehicle (2% Tween 80/0.5% methylcellulose suspension) or PAI-039, male beagle dogs were subjected to left circumflex (LCX) coronary artery injury via an intravascular electrode. Based on the pharmacokinetics of PAI-039 and a scheduled protocol.

Platelet Aggregation and Bleeding Time. To assess ex vivo platelet reactivity, blood (10 ml) was withdrawn from the right femoral vein into a plastic syringe containing 3.7% sodium citrate as the anticoagulant [1:10 citrate to blood (v/v)]. Platelet-rich plasma (PRP) was obtained by collecting the supernatant from whole blood centrifuged at 1400g for 10 min. Subsequently, platelet-poor plasma was prepared from the same blood sample by further centrifugation at 2000g for 15 min. Ex vivo platelet aggregation was assessed at 37°C with a four-channel platelet aggregometer (Bio-Data-PAP-4; Bio Data, Hatboro, PA) by recording the increase in light transmission through a stirred suspension of PRP. Aggregation was induced with 20 μM ADP, 4 μM U46619, and 2.5 μg/ml collagen. A subaggregatory dose of epinephrine (550 nM) was used to prime the platelets before the agonists were added. Bleeding times were determined with the use of a Surgicutt device (International Technidyne Corporation, Edison, NJ), which made a uniform incision 5 mm in length and 1 mm in depth on the upper surface of the tongue. The lesion was blotted with filter paper every 20 s until the transfer of blood to the filter paper ceased.

* Assess bleeding time, platelet aggregation, aPTT and PT
† Assess plasma concentration of platelet aggregation, aPTT and PT
‡ Assess ex vivo clot lysis activity
# Assess plasma PAI-1 activity

![Fig. 1](https://example.com/figure1.png)

Fig. 1. Diagrammatic representation of the experimental protocol used to investigate the effects of PAI-039 on coronary artery thrombosis. Hemodynamic parameters, including heart rate, blood pressure, and LCX coronary artery blood flow, were monitored throughout the protocol.
**APTT and PT Determination.** Activated partial thromboplastin time and prothrombin time were determined using an ST4 Coagulation Instrument (Diagnostica Stago, Asnières, France) with a four-channel clot detection system. Each APTT assay was run according to the reagent kit PTT Automate with addition of plasma to a reconstituted PTT Automate reagent and 0.025 M CaCl₂ (Diagnostica Stago). Each PT assay was run according to the reagent kit Neoplastine with addition of plasma to a reconstituted thromboplas- tin reagent (Diagnostica Stago). Citrated whole blood was withdrawn from the right femoral vein at the time points specified in Fig. 1. Sodium citrate (3.7%) was used as the anticoagulant [1:10 citrate to blood (v/v)]. Plasma was prepared by centrifugation at 2000g for 15 min. APTT and PT were measured using prepared reagents according to instrument directions.

**Plasma PAI-1 Activity Assay.** PAI-1 activity was determined using a two-stage indirect back titration method as described by Chandler et al. (1997). In the first stage, a fixed amount of PAI-1 (50 ng/ml) was added to a 75-µl aliquot of citrated plasma that reacts with the tPA present. In the second stage, residual tPA activity was determined by measuring the change in absorbance at 405 nm of 1 mM Spectrozyme tPA (American Diagnostica, Greenwich, CT), with the amount of color developed proportional to the amount of tPA in the sample. The endogenous PAI-1 activity in the plasma is equivalent to the difference in tPA activity with or without the addition of PAI-1 and was compared between plasma samples obtained from control or drug-treated dogs at 3 h postelectrolytic injury.

**Ex Vivo Plasma Clot Lysis Assay.** Rates of clot lysis were determined in samples collected 2 h after electrolytic injury using the methods described by Robbie et al. (2000). Plasma (100 µl) was added to a 96-well plate, followed by the addition of various concentrations of human recombinant tPA and incubated at room temperature for 5 min. Clot formation was triggered by the addition of bovine thrombin (10 U/ml) dissolved in CaCl₂. Lysis of the clot was measured by monitoring the change in absorbance at 405 nm at the time points specified in Fig. 1. Sodium citrate (3.7%) was used as the anticoagulant [1:10 citrate to blood (v/v)]. Plasma was prepared by centrifugation at 2000g for 15 min. APTT and PT were measured using prepared reagents according to instrument directions.

**Specificity Assays.** PAI-039 was assessed in vitro for potential inhibitory activity against structurally related serpins and serine proteases. PAI-039 was dissolved in dimethyl sulfoxide at a final concentration of 10 mM and then diluted 200× in buffer containing 50 mM Tris base, 150 mM NaCl, 10 µg/ml bovine serum albumin, and 0.01% Tween 80, pH 7.5. The effect of PAI-039 on the inhibition of α₁-antitrypsin (Lawrence and Loskutoff, 1986), antithrombin III (Bjork et al., 1992), α₂-antiplasmin (Wang et al., 2003), and tPA and tPA (Crandall et al., 2004) was assessed at concentrations of the compound ranging from 2.5 to 25 µM. In addition, the effect of PAI-039 on other coagulation proteases was tested using purified human enzymes (Enzyme Research Laboratories Inc., South Bend, IN) and synthetic peptide substrates in kinetic assays. Buffer (for 100% activity control) or PAI-039 was incubated with enzyme for 10 min at ambient temperature, followed by the addition of substrate. The initial rate of substrate cleavage was measured on a Spectromax Plus 384 or Gemini EM plate reader ( Molecular Devices, Sunnyvale, CA). Thrombin and Factor Xa assays were performed with a final concentration of 1 nM enzyme and 300 µM S-2366 or 150 µM S-2765, respectively (DiaPharma Group, West Chester, OH), in 50 mM Tris-

**Statistical Analysis.** The data are expressed as mean ± S.E.M. Time to thrombosis and thrombus weight between control and PAI-039-treated dogs were compared using a one-way ANOVA followed by Dunnett’s post hoc test. Total volume of blood flow after initial thrombotic occlusion was calculated from the area under the curve of the coronary blood flow over time. Area was determined using the measurements function in the Ponemah physiograph software. Area was converted to total blood flow by calculating the area of a 1-min tracing at 10 ml/min using the calibration feature of the flowmeter. Total blood flow during spontaneous reperfusion was compared with control using a one-way ANOVA followed by Dunnett’s post hoc test. Any change in blood flow >0.5 ml/min after sustained zero blood flow that lasted longer than 1 min was recorded as an episode of spontaneous reperfusion. Mean bleeding time, percentage of platelet aggregation, ex vivo clot lysis time, PAI-1 inhibited-tPA activity, and APTT and PT in control and PAI-039-treated dogs were compared with each group’s respective baseline using a one-way ANOVA followed by Dunnett’s post hoc test. Statistical significance was reported when p < 0.05. Heart rate and mean arterial blood pressure in the dog were compared using a repeated measures one-way ANOVA.

**Results**

PAI-039 was tested for in vivo oral efficacy in a canine model of coronary thrombosis by dosing 90 min before initiation of vascular injury. As shown in Table 1, PAI-039 pro-

### TABLE 1

Incidence and time to coronary occlusion after treatment with PAI-039

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incidence of Coronary Artery Occlusion</th>
<th>Coronary Artery Time to Occlusion</th>
<th>Incidence of Spontaneous Reperfusion (clot lysis)</th>
<th>Thrombus Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (n = 5)</td>
<td>6/6 (100%)</td>
<td>31.7 ± 6.3</td>
<td>0/6 (0%)</td>
<td>7.6 ± 1.5</td>
</tr>
<tr>
<td>PAI-039 (1 mg/kg, n = 5)</td>
<td>5/5 (100%)</td>
<td>34.6 ± 4.1</td>
<td>3/5 (60%)</td>
<td>8.7 ± 0.6</td>
</tr>
<tr>
<td>PAI-039 (3 mg/kg, n = 5)</td>
<td>5/5 (100%)</td>
<td>66.0 ± 6.4*</td>
<td>3/5 (60%)</td>
<td>8.8 ± 1.3</td>
</tr>
<tr>
<td>PAI-039 (10 mg/kg, n = 6)</td>
<td>6/6 (100%)</td>
<td>56.7 ± 7.4*</td>
<td>4/6 (67%)</td>
<td>3.6 ± 1.0*</td>
</tr>
</tbody>
</table>

*p Statistically significant difference compared with vehicle (p < 0.05).
longed the time to coronary occlusion when dosed at both 3 and 10 mg/kg, compared with vehicle-treated controls. After complete coronary thrombosis, 60% of dogs treated with 1 and 3.0 mg/kg PAI-039 and 67% of dogs treated with 10 mg/kg PAI-039 exhibited spontaneous coronary reperfusion, whereas no measurable blood flow was recorded for the control group throughout the remainder of the experiment. To quantitate the degree of reperfusion in each animal, the area under the coronary blood flow curve was measured over time. The area (total volume of blood flow) was assessed after the initial occlusion ($p < 0.05$ compared with control). After treatment with 3 or 1 mg/kg PAI-039, spontaneous reperfusion resulted in 26.0 ± 11.7 ml of blood flow after the initial occlusion ($p < 0.05$ compared with control). After treatment with 3 or 1 mg/kg PAI-039, spontaneous reperfusion resulted in 26.0 ± 7.1 and 2.8 ± 2.4 ml of blood flow, respectively. The 10-mg/kg treatment group also exhibited reduced thrombus weight. A schematic representation of coronary artery reperfusion/reocclusion pattern for the 10-mg/kg group is shown in Fig. 2. Mean arterial blood pressure and heart rate were not affected by the administration of PAI-039 (data not shown).

**Effect of PAI-039 on Platelet Aggregation, Bleeding Time, PT, and APTT.** Ex vivo platelet responses were measured throughout the experiment. PRP prepared from blood of control dogs showed typical ex vivo aggregation responses to 20 μM ADP, 4 μM U46619, and 2.5 μg/ml collagen over the entire course of the experiment (Table 2). PAI-039 treatment at 10 mg/kg had no effect on platelet responses to any of these agonists, indicating that reperfusion in drug-treated groups was not related to inhibition of these common platelet activation pathways. Similar responses were observed at the 1- and 3-mg/kg dose (data not shown). The additional hemostatic measurements of APTT, PT, and template bleeding time were also determined throughout the experiment. At the highest dosage of PAI-039, there was no observed effect on template bleeding time, APTT, or PT (Table 3). Similar results were obtained at the lower doses (data not shown).

**Plasma PAI-1 Activity.** The PAI-1 inhibitory effect of PAI-039 was determined in citrated plasma at 3 h postelectrolytic injury. As shown in Fig. 3, each dosage of PAI-039 reduced plasma PAI-1 activity, with statistical significance observed in the 10-mg/kg group ($p < 0.05$).

**Ex Vivo Clot Lysis.** Plasma clots prepared from blood sampled after coronary thrombosis were compared between control and drug-treated groups for rates of lysis. The assay performance was initially characterized by determining lysis across a range of concentrations of exogenously added tPA, ranging from 30 to 1000 ng/ml, with rates of lysis correlating to the tPA concentration. A representative data set from the 1-mg/kg treatment group at two different concentrations of exogenous tPA (125 and 250 ng/ml) is shown in Fig. 4, indicating that although turbidity is similar between groups at 5 min, the rate of clot lysis is accelerated significantly in PAI-039-treated dogs compared with controls ($p < 0.05$; repeated measures two-way ANOVA). For this data set, the time to 50% clot lysis in the control group was 2821 ± 201 s with 250 ng/ml tPA, which was accelerated to 1482 ± 274 s with 250 ng/ml tPA. This value was further accelerated to 719 ± 390 s in samples from dogs treated with 1 mg/kg PAI-039. For the other treatment groups, the time to 50% clot lysis was 767 ± 289 s (3 mg/kg PAI-039) and 734 ± 238 s (10 mg/kg PAI-039). These data are in agreement with the peripheral plasma reduction in PAI-1 activity.

**Plasma Concentration and Pharmacokinetics of PAI-039.** PAI-039 was dosed intragastrically after induction of anesthesia in the electrolytic injury experiments. The mean plasma value at the 3-mg/kg dose was 0.575 ± 0.07 μg/ml for the 6-h experiment, with an AUC_0–6 of $3.1 \mu g \cdot h/ml$. With a molecular weight of 439, the mean concentration of PAI-039 at 3 h after dosing was approximately 1.3 μM. This concentration is near the previously reported IC_{50} of 2.7 μM (Elodah et al., 2004). Three hours postinjury, the plasma concentration in the 3-mg/kg group was 0.630 ± 0.168 μg/ml and in the 10-mg/kg group was 1.021 ± 0.378 μg/ml.

**TABLE 2**

<table>
<thead>
<tr>
<th>Agonist and Treatment</th>
<th>Baseline</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP (20 μM)</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>66.2 ± 4.4</td>
<td>70.2 ± 2.9</td>
<td>68.3 ± 3.5</td>
<td>62.8 ± 6.5</td>
</tr>
<tr>
<td>PAI-039 (10 mg/kg)</td>
<td>75.8 ± 4.3</td>
<td>73.8 ± 1.6</td>
<td>74.3 ± 3.8</td>
<td>77.2 ± 5.8</td>
</tr>
<tr>
<td>U46619 (4 μM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>63.2 ± 7.2</td>
<td>71.8 ± 10.2</td>
<td>71.6 ± 1.7</td>
<td>66.2 ± 2.7</td>
</tr>
<tr>
<td>PAI-039 (10 mg/kg)</td>
<td>66.2 ± 10.0</td>
<td>52.8 ± 10.6</td>
<td>56.3 ± 8.9</td>
<td>72.5 ± 10.9</td>
</tr>
<tr>
<td>Collagen (2.5 μg/ml)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>81.6 ± 4.9</td>
<td>73.6 ± 2.4</td>
<td>77.0 ± 3.2</td>
<td>71.2 ± 4.9</td>
</tr>
<tr>
<td>PAI-039 (10 mg/kg)</td>
<td>79.2 ± 4.2</td>
<td>77.5 ± 8.2</td>
<td>78.3 ± 5.4</td>
<td>77.8 ± 2.1</td>
</tr>
</tbody>
</table>

Values represent mean ± S.E.M. percentage of aggregation for $n = 5$ to 6 experiments.
In addition to measuring plasma concentrations at 3 h after dosing, a full pharmacokinetic profile of PAI-039 was assessed under good laboratory practice conditions in the conscious dog. The mean plasma concentrations of PAI-039 after a single 3-mg/kg oral dose or 1 mg/kg i.v. dose are shown in Fig. 5. The observed mean (± S.D.) peak plasma concentration (C_{max}) for PAI-039 after oral administration was 2125 ± 937 ng/ml and was observed at 1.8 h after dosing, which was higher than the value observed in the anesthetized dogs at the same dose. The C_{min} value after i.v. dosing was 6863 ± 1234 ng/ml. The mean (± S.D.) AUC_{0-\infty} values for PAI-039 were 17,818 ± 9047 ng · h/ml after oral dosing and 13,274 ± 3799 ng · h/ml after i.v. dosing. The mean (± S.D.) clearance (CL_{F}) and the steady-state volume of distribution (Vd_{ss}) after i.v. dosing were 0.0800 ± 0.0216 l/kg and 0.340 ± 0.130 l/kg, respectively. The mean (± S.D.) bioavailability of PAI-039 was 43.9 ± 15.3%. The mean (± S.D.) t_{1/2} values of PAI-039 were 6.2 ± 1.3 h after i.v. dosing and 6.2 ± 0.8 h after oral dosing.

Specificity. At 25 μM, PAI-039 exhibited no inhibitory activity against any of the closely related serpins or serine proteases assayed. This concentration is approximately 10× greater than the IC_{50} for PAI-039 against PAI-1 and also approximately 25× greater than the plasma levels of the compound observed 3 h after dosing in the 3-mg/kg group and 10× the concentration observed at 10 mg/kg. Determination of potential inhibitory effects at higher concentrations was limited by the critical micelle concentration of the compound. These data indicate specificity for PAI-039 against PAI-1, which was further supported by a lack of interaction of PAI-039 against a variety of targets as published previously (Elokdah et al., 2004).

**Table 3**

<table>
<thead>
<tr>
<th>Measure and Treatment</th>
<th>Baseline</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bleeding time (s)</strong></td>
<td>121.4 ± 9.8</td>
<td>110.6 ± 14.8</td>
<td>122.8 ± 18.3</td>
<td>89.0 ± 21.2</td>
</tr>
<tr>
<td>Control</td>
<td>119.0 ± 20.9</td>
<td>111.3 ± 9.8</td>
<td>92.0 ± 9.4</td>
<td>108.5 ± 6.6</td>
</tr>
<tr>
<td>PAI-039 (10 mg/kg)</td>
<td>14.6 ± 0.8</td>
<td>13.7 ± 0.6</td>
<td>14.3 ± 0.3</td>
<td>12.8 ± 0.6</td>
</tr>
<tr>
<td><strong>APTT (s)</strong></td>
<td>14.0 ± 0.8</td>
<td>13.6 ± 0.6</td>
<td>14.8 ± 1.9</td>
<td>12.9 ± 1.0</td>
</tr>
<tr>
<td>Control</td>
<td>22.8 ± 0.9</td>
<td>21.3 ± 0.7</td>
<td>22.8 ± 1.1</td>
<td>21.6 ± 0.7</td>
</tr>
<tr>
<td>PAI-039 (10 mg/kg)</td>
<td>22.2 ± 0.3</td>
<td>22.1 ± 0.8</td>
<td>23.4 ± 1.8</td>
<td>20.9 ± 0.4</td>
</tr>
</tbody>
</table>

**Fig. 3.** Effect of PAI-039 or vehicle on PAI-1 activity. Values are expressed as mean ± S.E.M. for n = 3 to 6 samples per group and represent the change in PAI-1 activity in control and drug-treated dogs 3 h after initiation of electrolytic injury. PAI-1 activity was reduced significantly in dogs administered 10 mg/kg PAI-039 compared with vehicle-treated dogs (p < 0.05). Comparisons were made using a one-way ANOVA followed by Dunnett’s post hoc test.

**Fig. 4.** Ex vivo plasma clot lysis in plasma from dogs administered PAI-039 (1 mg/kg p.o.) or vehicle. Clots were prepared with a final tPA concentration of 125 or 250 ng/ml. Lysis of the clot was seen as a decrease in absorbance over time. Data are expressed as the mean percentage of lysis from five different dog plasma samples. The rate of clot lysis is accelerated significantly in PAI-039-treated dogs compared with controls (p < 0.05; repeated measures two-way ANOVA).

**Discussion**

Although PAI-1 is the most important physiological inhibitor of both tPA and uPA, an orally active series of small molecule PAI-1 inhibitors has not been previously identified. PAI-1 as a target for drug development has been pursued, and the status of small molecule inhibitors was recently reviewed, which includes diverse chemical structures often lacking oral bioavailability, in vivo efficacy, and an obvious structure-activity relationship (Wu and Zhao, 2002). PAI-039 is the second in a series of orally active PAI-1 inhibitors that we have identified by high throughput screening and structure-based medicinal chemical synthesis (Crandall et al., 2004; Elokdah et al., 2004). The screening format was designed to specifically account for the multiple conformations of PAI-1 (Lawrence, 1997) and to identify molecules with higher binding affinity for the active rather than the latent form.
is dose dependently prolonged by PAI-039, but not prevented.

The other indices of PAI-1 inhibition, plasma PAI-1 and clot lysis time, were also positively affected by PAI-039 treatment, but with less separation between doses. Because PAI-1 has a short plasma half-life due to the metastable conformation of the active form, PAI-1 inactivation would occur by two additive mechanisms, namely, spontaneous conversion to the latent conformation and pharmacological inhibition by PAI-039. The inactivation of concentrated platelet PAI-1 at the site of the coronary lesion may therefore not be equally reflected by PAI-1 values in the peripheral blood. This hypothesis is supported by recanalization studies in mice reported by Zhu et al. (1999), where an infusion of tPA was more effective in restoring blood flow in the PAI-1 null than wild-type mice, despite producing plasma levels many times that of circulating PAI-1. Additionally, in a recent in vivo study in humans, the regulation of local fibrinolysis was not accurately reflected by peripheral plasma levels of PAI-1 and tPA (HrãAnkneldottir et al., 2004). Ultimately, these data suggest that a functional endpoint, such as coronary blood flow, is required in addition to biochemical measurements in peripheral blood to truly establish efficacy of PAI-1 inhibitors.

Endogenous PAI-1 inhibition allows active availability of unbound circulating tPA and acceleration of fibrinolysis. Accordingly, the initial clinical indication for a PAI-1 inhibitor would be similar to that of thrombolytic agents, including treatment of acute myocardial infarction and peripheral arterial occlusion (Verstraete, 1998; Armstrong and Collen, 2001). Since the elevation in circulating PAI-1 has been shown to contribute to failure of tPA to stimulate peripheral arterial thrombolysis (Nicholls et al., 2003), the use of a PAI-1 inhibitor together with a lower dose of tPA could prove both effective and reduce the risk of intracerebral hemorrhage and bleeding observed with standard tPA therapy alone. In addition to indications in acute settings, it is important to note that PAI-1 is elevated in chronic diseases such as type 2 diabetes and atherosclerosis (Festa et al., 2002; Sobel et al., 2003), and preclinical studies suggest that PAI-1 inhibition may be beneficial in their treatment (Eitzman et al., 2000, Ma et al., 2004). Finally, PAI-1 is regulated by proinflammatory cytokines, which may further contribute to insulin resistance and vascular risk (Juhan-Vague et al., 2003). Inhibition of PAI-1 may therefore have potential therapeutic benefit in both acute and chronic diseases. The current study provides evidence of preclinical utility in acute coronary thrombosis. Future experiments will focus on the potential beneficial effect of PAI-039 in models of chronic cardiovascular and metabolic disease.

Acknowledgments

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References


Carmeliet P, Stassen JM, Schoonjans I, Ream B, van den Oord JJ, De Mol M,


