Resolution of venous thrombosis using a novel oral small-molecule inhibitor of P-selectin (PSI-697) without anticoagulation

Daniel D. Myers, Jr.1,2, Shirley K. Wrobleski1, Chris Longo1, Patricia W. Bedard4, Neelu Kaila4, George D. Shaw4, Frank J. Londy3, Suzan E. Rohrer3, Beverly A. Fex1, Paul J. Zajkowski1, Thomas R. Meier1,2, Angela E. Hawley1, Diana M. Farris1, Nicole E. Ballard1, Peter K. Henke1, Robert G. Schaub4, Thomas W. Wakefield1

1Jobst Vascular Research Laboratories, Section of Vascular Surgery, 2Unit for Laboratory Animal Medicine and 3Department of Radiology, University of Michigan Medical Center, Ann Arbor, Michigan, USA; 4Wyeth Research, Cambridge, Massachusetts, USA

Summary
P-selectin inhibition has been shown to decrease thrombogenesis in multiple animal species. In this study, we show that a novel oral small-molecule inhibitor of P-selectin, PSI-697, promotes thrombus resolution and decreases inflammation in a baboon model of venous thrombosis. Experimental groups consisted of the following: 1) primates receiving a single oral dose of PSI-697 (30 mg/kg) daily starting three days pre-iliac vein balloon occlusion, and continued for six days; 2) primates receiving a single treatment dose of a low-molecular-weight-heparin (LMWH) (1.5 mg/kg) daily starting one day pre-iliac balloon occlusion, and continued for six days; and 3) primates receiving a single oral dose of a vehicle control daily starting three days pre-iliac vein balloon occlusion, and continued for six days. Animals receiving PSI-697, although thrombosed after balloon deflation, demonstrated greater than 80% vein lumen opening over time, with no opening (0%) for vehicle control (p<0.01). LMWH opening evidence after balloon deflation slightly deteriorated over time compared to PSI-697. PSI-697 therapy also significantly decreased vein wall inflammation determined by magnetic resonance venography (MRV). Importantly, this beneficial opening occurred without measured anticoagulation. Animals receiving PSI-697 demonstrated significantly increased plasma D-dimer levels versus LMWH and control animals six hours post thrombus induction (p<0.01). This study is the first to demonstrate the effectiveness of oral P-selectin inhibition to modify venous thrombogenesis, increase vein lumen opening, and decrease inflammation in a large animal model.

Keywords
Deep venous thrombosis, thrombus resolution, small-molecule inhibitor of p-selectin, inflammation, animal models

Introduction
Deep venous thrombosis (DVT) is a significant national health problem in the general population. Treatment costs for this disease in the United States exceed billions of dollars annually just for the acute treatment of venous thrombosis, not including therapy for the sequelae of DVT such as chronic venous insufficiency, pulmonary embolism (PE) and subsequent chronic pulmonary hypertension (1, 2). Recently, venous thromboembolism, including DVT and PE, has been estimated to occur in 450,000 recognized cases with a total of recognized and unrecognized cases to be 1,350,000 annually (3). Chronic venous insufficiency affects between 400,000 and 500,000 patients with skin ulcerations and six to seven million patients with manifestations of chronic venous disease including valvular dysfunction and is often the result of venous thrombosis (4–11).

In 1856, Virchow hypothesized the genesis of intravascular thrombosis which includes blood stasis, changes in the vessel...
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wall, and changes in the blood leading to a prothrombotic state (12). It was not until a century later that the interrelationship between thrombosis and inflammation was seriously considered (13, 14). More recently, the selectins, especially P-selectin, have been identified as having an important role in the pathogenesis of both venous and arterial thrombosis (15–23). Selectins are the first up-regulated glycoproteins during an inflammatory response; they are expressed by activated platelets (α-granule release) and endothelial cells (Weibel-Palade body release) (24–28). P-selectin glycoprotein ligand-1 (PSGL-1), the receptor for P-selectin, is expressed on the majority of circulating leukocytes. P-selectin:PSGL-1 binding promotes leukocyte-leukocyte, leukocyte-platelet, and leukocyte-endothelial cell interactions (29–31). These interactions support a prothrombotic environment triggering a number of cell signaling pathways and releasing prothrombotic microparticles (32–35). There have been a number of approaches taken to inhibit P-selectin mediated prothrombotic and pro-inflammatory effects including monoclonal antibodies, recombinant soluble receptor antagonists, and carbohydrate-based small-molecule antagonists given intravenously (i.v.) or subcutaneously (s.c.) (16, 20, 22, 23, 36–38).

The current study evaluates the therapeutic potential of a novel tetrahydrobenzoquinoline salicylate oral small-molecule inhibitor of P-selectin (PSI-697) (37) in a large animal model of venous thrombosis. We elected to compare this agent with low-molecular-weight heparin (LMWH) at a treatment dose. Previously, we have shown that PSI-697 effectively decreases thrombogenesis in a murine model of stasis-induced thrombosis (39), and decreases vein wall fibrosis in a rat model of stenosis-induced inferior vena cava (IVC) thrombosis (38). In this study we hypothesized that the oral small-molecule inhibitor of P-selectin (PSI-697) would decrease thrombosis and inflammation in a baboon model of stasis-induced venous thrombosis. An orally administered anti-thrombotic without direct anticoagulant activity, has potentially significant advantages over i.v. or s.c. administration of anti-coagulant therapies for the prevention and treatment of DVT.

Material and methods

P-selectin inhibition

The small-molecule inhibitor of P-selectin (PSI-697, Wyeth Research, Cambridge, MA, USA) is an orally available, tetrahydrobenzoquinoline salicylate inhibitor of P-selectin. This compound has been demonstrated in vitro to disrupt the binding of P-selectin to its ligand P-selectin glycoprotein ligand-1 (PSGL-1), and reduce white cell rolling in the microvasculature of mice (36). This oral formulation has been shown to significantly decrease thrombosis in a mouse model of venous thrombosis and to decrease vein wall fibrosis in a rat model of venous thrombosis (38).

Primate balloon-occlusion iliac vein stasis model

Male juvenile baboons (Papio anubis) underwent iliac vein thrombosis with temporary six-hour balloon occlusion as previously described (20, 40). Seven juvenile baboons were evaluated for nine experiments. In two primates, both iliac veins were evaluated one month apart to insure drug clearance. Experimental groups consisted of the following (see Table 1): 1) primates receiving a single oral dose of PSI-697 (30 mg/kg, orally, n=3) in hollow marshmallow circus peanut (Spangler Candy Co., Bryan, OH, USA) capped with jelly daily starting three days pre-iliac vein balloon occlusion, and continued for six days. One additional primate was administered PSI-697 orally at the same above mentioned dose but in a different vehicle (placed in a banana). This change of vehicle resulted in both a dosing and experimental failure; thus, this primate was not included in the study analysis; 2) Primates receiving a single treatment dose of Lovenox® enoxaparin sodium injection (LOV) (1.5 mg/kg, treatment dose in humans, s.c. injection, n=3) daily starting one day pre-iliac balloon occlusion, and continued for six days; and 3) primates receiving a single oral dose of a vehicle control (n=3) daily starting three days pre-iliac vein balloon occlusion, and continued for six days. Drug treatment was stopped at day 6 post balloon occlusion in all animals. All animals underwent iliac vein thrombosis on day 0. Ultrasound imaging at time of balloon-catheter removal at T+6 hours was performed. Primates were then re-evaluated on days 2 and 6. Additionally, the lungs were evaluated by magnetic resonance imaging and no evidence of PE was found at any time point.

The dose of 30 mg/kg/day PSI-697 was based on the effective oral dose determined by dosing studies in primates at Wyeth Research and the Jobst Vascular Research Laboratories. The LMWH Lovenox® dose (1.5 mg/kg) is the anti-coagulant treatment dose for clinical treatment of DVT. We chose to use an anti-coagulant dose due to the fact that the PSI-697 dose would be the same for either prophylaxis or treatment applications and we wanted to test PSI-697 against the most rigorous low-molecular-weight dosage possible. Both PSI-697 and LOV compounds were in the circulation at the baseline time points in this study.

Contrast venography/thrombus analysis

Venography was performed using a 22-gauge i.v. catheter placed bilaterally into the saphenous veins of primates. Five-ml Hypaque diatrizoate meglumine (USP, 60%; Nycomed, Inc, Princeton, NJ, USA) was injected into both legs simultaneously. Imaging was performed with a BV29 C-arm fluoroscopy unit (Phillips Medical System, Cincinnati, OH, USA); images were stored on computer. All animals at each time point had venograms performed both in anteroposterior and lateral projection.

Duplex ultrasound analysis

Duplex ultrasound was used to determine both the presence of thrombus and iliac valve function (the primate has a valve in the mid-iliac vein). For thrombus evaluation, color duplex ultrasound imaging was performed using a Siemens Sonoline Antares (Siemens Medical Systems, Inc., Issaquah, WA, USA), or a GE® Logic 700 (General Electric, Milwaukee, WI, USA). A linear multi-hertz (7.5 MHz) transducer was utilized. Thrombus was

Table I: Experimental animal numbers.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>T+6 hours</th>
<th>Day 2</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>n=3</td>
<td>n=3</td>
<td>n=3</td>
<td>n=3</td>
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<td>n=3</td>
<td>n=3</td>
<td>n=3</td>
<td>n=3</td>
</tr>
<tr>
<td>LOV</td>
<td>n=3</td>
<td>n=3</td>
<td>n=3</td>
<td>n=3</td>
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</tbody>
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evaluated by both lack of compression, lack of flow, and the presence of a dilated vein void of echoes (acute) or a shrunken vein full of echoes (chronic), typical criteria used for clinical imaging. The same ultrasound units were used for the valve function analysis. For valve function, a proximal abdominal compression technique combined with ultrasound analysis was used. A normal iliac valve closure time is defined as a valve closing ≤500 milliseconds (ms) after compression.

### Magnetic resonance imaging – time-of-flight and gadolinium-enhanced

Magnetic resonance venography (MRV) was performed using a 1.5-T MR imaging system (Singa Horizon LX, General Electric Medical Systems, and Milwaukee, WI, USA) with 0.1 software. Thrombosis was quantitated by time-of-flight (TOF) imaging, in which the flow channel area was quantitated from one exam to the next and percent vein opening determined by drawing a region of interest around the flow channel, the contralateral un-involved iliac vein, and then calculating a ratio of open vein (flow) to the contralateral unaffected side. Inflammation was quantitated using gadolinium (Gd) administration with MRV. Gd is a heavy metal chelate that prolongs the T1 relaxation time of blood, and extravasates selectively into areas with capillary leak and as such is a non-invasive marker of inflammation. Post-Gd images were acquired of the left and right iliac veins. Quantitative analysis involved drawing a region of interest (ROI) around the area of Gd-enhancement and measuring the area in mm². Values obtained from the combined proximal and distal iliac vein segments of each primate were used for statistical analysis. The evaluation for PE was done by Gd-enhanced magnetic resonance imaging using a previously described quiet breathing technique (41).

### Microparticle analysis

Circulating microparticle populations were evaluated as previously described (42). In brief, 4.5 ml of whole blood was removed by vein puncture using a syringe primed with 10% acid citrate dextrose (ACD). Platelet-poor plasma (PPP) was obtained by centrifuging blood at 1,500 g and 4°C for 25 minutes (min) and centrifuged once more for 2 min at 15,000 g. PPP (200 µl) obtained from each primate was diluted 1:3 with 600 µl of HEPES buffer [10 mM HEPES/5 mM KCl/1 mM Mg /136 mM NaCl (pH 7.4)]. Samples were centrifuged for 2 hours (h) at 200,000 g. Pelleted microparticles were resuspended in HEPES buffer. Antibodies evaluated included PE anti-Human CD41a (stains platelets) 10 µl (BD Pharmingen, San Diego, CA, USA) or control mouse PE IgG (BD Pharmingen), and FITC anti-Human CD11b (stains neutrophils) 1 µl (Caltag, Burlingame, CA, USA), or control mouse FITC IgG (BD Pharmingen). Test samples were stored on ice, washed, and then centrifuged for 1 h at 200,000 g, fixed with 0.5% paraformaldehyde prior to fluorescence-activated cell scanning (FACS) analysis. For microparticle quantification, a known quantity (250,000) of 3.0 to 3.4 µm fluorescent beads (SPHERO™, BD Pharmingen) was added to each sample, and acquisition was stopped after 50,000 bead events were counted. These counts represented 20% of the total bead and microparticle populations combined. Circulating microparticle populations were determined by evaluating thrombus plot quadrant numbers and multiplied by a factor of five to correct for initially counting only 20% of the total microparticle population (total number of microparticles events counted). Flow cytometry analysis was performed by the Biomedical Research Core Facilities (University of Michigan Medical Center, Ann Arbor, MI, USA) on a Becton Dickinson FACS Vantage SE cell sorter with Cell Quest (Becton Dickinson, MAC platform) using forward versus side scatter to gate on microparticle populations.

### D-dimer analysis

D-dimer analysis was performed using a latex-enhanced automated semi-qualitative turbidometric assay, in which 20 µl of sodium-citrate non-human primate plasma was evaluated. This assay measures circulating fibrin derivatives containing D-dimers (Dade-Behring, Deerfield, IL, USA). All tests were performed according to the manufacturer’s recommendations with data reported in µg/ml. Positive and negative controls were included in each batch to ensure a reliable test system. Serial dilutions included 1:2, 1:4, and 1:8 plasma diluted with Dimentest® buffer. Positive tests were defined as plasma D-dimer concentrations ≥0.20 µg/ml.

### Coagulation tests and hematological analysis

Coagulation studies included activated partial thromboplastin time (aPTT), thrombin clotting times (TCT; Dade Diagnostics, Miami, FL, USA), and template bleeding times (Allegiance Health Care Corp, McGaw Park, IL, USA). Circulating fibrinogen levels were measured in plasma samples using a fibrinometer (Bectin Dickinson, Cockeysville, MD, USA) with standardized test reagents (Dade Behring, Marburg, Germany). White blood cells and platelet population analysis were performed on an automated Hema VET®, (CDC technologies, Inc., Oxford, CT, USA).

### Drug levels

All primates receiving PSI-697 had plasma samples evaluated for drug concentrations (70 to 105 minutes [min]) post oral dosing. Plasma levels of test compounds were determined by high-pressure liquid chromatographic-tandem mass spectrometric (LC/MS-MS) assay (Wyeth Research). A log trapezoid non-compartmental analysis was used in pharmacokinetic analysis. A photometric test (Chromogenix, DiaPharma Group, Inc., West Chester, OH, USA) was used to determine plasma levels of Lovenox® enoxaparin sodium 180 min post dosing by s.c. injection.

### Statistical evaluation and animal use

Statistical analysis included mean ± SEM, analysis of variance (ANOVA), and unpaired Student t-tests for parametric data (SPSS Sigma Stat 2.03, Aspire Software International, Leesburg, VA, USA). Significance was defined as p≤0.05. The health status of all animals was monitored, and all animals were free of pathogens. All non-human primates were housed and cared for by the University of Michigan Unit for Laboratory Animal Medicine. The University of Michigan is an AAALAC (Association for the Assessment and Accreditation of Laboratory Animal Care International)-accredited facility under the direction of a veterinarian according to the “Principles of Laboratory Animal
Results

PSI-697 increases vein opening over time
Combined proximal and distal segments from each primate were evaluated. At the 6-h time point (balloon deflation), all control and PSI-697 animals demonstrated large thrombi by ultrasound, while the LOV animals did not form a clot (Fig. 1). However, animals receiving PSI-697, compared to controls demonstrated an increase in vein opening by ultrasound and significant increase in vein opening by magnetic resonance venography at day 2 (78 ± 10% vs. 0 ± 0%, p<0.01) and day 6 (78 ± 8% vs. 0 ± 0%, p<0.01). Animals receiving LOV did not develop thrombosis at the time of balloon occlusion and demonstrated significant vein opening at day 2 (82 ± 7% vs. 0 ± 0%, p<0.01) and day 6 (70 ± 8% vs. 0 ± 0%, p<0.01) compared to controls (Fig. 2).

The presence or absence of thrombus was assessed by both ultrasound imaging and contrast venography (Figs. 1 and 3). Note the representative series of contrast venogram images from animals from each group through day 6 (Fig. 3). No pulmonary emboli were noted in any of the groups at each time point, as measured by magnetic resonance venography.

PSI-697 and LMWH therapy decreases vein wall inflammation
PSI-697 significantly decreased vein wall inflammation as determined by magnetic resonance venography at day 6 compared to controls (11 ± 3 mm² Gd enhancement vs. 28 ± 4 mm² Gd enhancement, p<0.01) (Fig. 4). The LOV animals also demon-
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stratified a significant decrease in vein wall inflammation at day 6 compared to controls (9 ± 3 mm$^2$ vs. 28 ± 4 mm$^2$ Gd enhancement, p<0.01) (Fig. 4).

**PSI-697 maintains iliac valve function post thrombosis**

Ultrasound examination of the affected veins at each time point during the study indicated that all animals receiving PSI-697 maintained normal valve closure times, while animals receiving LMWH (LOV) also maintained normal valve closure times. Control animals with marked iliac vein thrombosis had abnormal valve function with prolonged valve closures.

**PSI-697 decreases circulating microparticle populations**

We decided to combine time points of the T+6 hours, day 2, and day 6 to emphasize trends in microparticle data, when it became clear that no statistical differences existed at individual time points. PSI-697 therapy showed a trend in decreasing circulating microparticle populations post balloon occlusion compared to controls and animals given LOV (Table 2). Animals receiving PSI-697 had the lowest fold increase from baseline of circulating leukocyte-derived, platelet-derived, and total microparticles between the groups (not significant). For total microparticles, combining the time points T+6 h, day 2 and day 6, there was a 1.76-fold increase compared to baseline in the control animals, a 1.55-fold increase compared to baseline in the LOV animals, and only a 1.07-fold increase in the PSI-697 treated animals. Of interest, this same relationship was more pronounced when evaluating leukocyte derived microparticles (2.26-fold increase vs. baseline in the control animals, a 2.27-fold increase vs. baseline in the LOV animals, with only a 1.19-fold increase in animals receiving PSI-697 therapy).

**Coagulation/hematology**

PSI-697 and LOV compounds were both in the circulation at the start of this study. Primates receiving LOV had a statistically significant increased aPTT versus animals treated with PSI-697 (38.5 ± 3 vs. 29.2 ± 2 seconds [s], p<0.05) at baseline (Table 3). No other significant differences in aPTT were noted between the groups during this study. Animals receiving LOV had a statistically significant increased TCT time versus controls at baseline (22.4 ± 0.3 vs. 17.2 ± 1 s, p<0.05) and at day 2 (39.6 ± 2 vs. 23.4 ± 2 s, p<0.01) (Table 3). At day 2, the LOV group had a signifi-

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**Figure 3:** Contrast venogram composite in representative animals of all groups, from baseline to day 6, post balloon catheter injury.

**Figure 4:** Vein wall inflammation was significantly decreased by PSI-697 therapy at day 6 (11 ± 3 mm$^2$ Gd enhancement vs. 28 ± 4 mm$^2$ Gd enhancement, p<0.01), compared to controls as determined by magnetic resonance venography. Vein wall inflammation was also significantly decreased by LOV therapy at day 6 (9 ± 3 mm$^2$ Gd enhancement vs. 28 ± 4 mm$^2$ Gd enhancement, p<0.05), compared to controls. Gd = gadolinium enhancement.
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significantly increased TCT compared to the PSI-697 group (39.6 ± 2 vs. 20.7 ± 1 s, p<0.01) (Table 3).

Bleeding times were evaluated for all experimental groups at the baseline through day 6 time points. Note the transient increase in the bleeding time at day 2 in the PSI-697 group (up to 6 min). This was due to one animal with a transient increase in bleeding time at day 2 that normalized by day 6. All bleeding times recorded in this study were within normal reference ranges for juvenile baboons previously evaluated in our laboratory using this thrombosis model (1.5 to 6 min). No other elevations in bleeding time were noted between the groups at any other time point (Table 3).

No significant changes were seen in fibrinogen levels at any time point between the experimental groups. A transient decrease in platelet counts was noted at both T+6 (time of balloon catheter removal) and at day 2 post balloon catheter occlusion in all groups (data not shown), which reverted to normal at day 6. These changes in platelet count were not due to technical error but were likely due to the surgical procedure itself. Experimental animals in any group did not present with bruising, bleeding, or wound healing complications during the course of this study.

**PSI-697 promotes early fibrinolysis**

Animals receiving PSI-697 had significantly increased plasma D-dimer levels (≥0.20 µg/ml) when compared to thrombosed controls 6 h post thrombus induction (0.6 ± 0 vs. 0 ± 0 µg/ml, p<0.01). Animal receiving LOV demonstrated D-dimer levels below 0.20 µg/ml at the same time point (p<0.01). On both days 2 and 6 post thrombus induction, all control and PSI-697 animals had elevated circulating plasma D-dimer levels. The LOV group had only one animal on day 2 and two animals on day 6 with elevated circulating D-dimer levels (Table 3).

**Drug levels**
The effective plasma concentration of PSI-697 was from 400 to 1,800 ng/ml (75 to 105 min post dosing) in this animal model. A single primate excluded from analysis received PSI-697 with a different vehicle which produced a non-effective plasma concentration (190 ng/ml) of the compound. This dosing failure prevented thrombus resolution in this single primate. Lovenox® enoxaparin sodium plasma concentrations were 0.6 IU/ml anti-Xa units 180 min post dosing in this study.

**Discussion**
The vascular anatomy of the baboon is similar to that of humans, which makes them an ideal comparative model to study vascular diseases. The baboon is an upright animal, like humans, and the lower extremity venous physiology is reasonably equivalent to that in man. Baboons infused experimentally with human plasma proteins and fibrinogen do not mount an immune response, thus...

### Table 2: Circulating microparticle population, fold changes from baseline as determined by FACS analysis.

<table>
<thead>
<tr>
<th>T+6 hours, day 2, day 6</th>
<th>Control</th>
<th>PSI-697</th>
<th>LOV</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEU microparticles</td>
<td>2.26 ± 1.03</td>
<td>1.19 ± 0.35</td>
<td>2.27 ± 0.71</td>
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<tr>
<td>PLT microparticles</td>
<td>0.97 ± 0.32</td>
<td>0.88 ± 0.32</td>
<td>0.93 ± 0.40</td>
</tr>
<tr>
<td>LEU-PLT microparticles</td>
<td>1.45 ± 0.51</td>
<td>1.25 ± 0.40</td>
<td>1.08 ± 0.36</td>
</tr>
<tr>
<td>Total microparticles</td>
<td>1.76 ± 0.68</td>
<td>1.07 ± 0.32</td>
<td>1.55 ± 0.45</td>
</tr>
</tbody>
</table>

**Table 3: Coagulation test.** Significance was defined as **p<0.05 for LOV versus PSI-697, aPTT, and *p<0.01 for LOV versus controls and PSI-697, TCT. Bleeding times in minutes (min) were within normal reference levels for control baboons previously evaluated in our laboratory (1.5 to 6 min, [19, 20]). Elevated plasma D-dimer levels were defined as ≥0.20 µg/ml. Significance was defined as *P<0.01 for animals receiving PSI-697 vs. controls, LOV 6 hours post thrombus induction. aPTT = partial thromboplastin time, TCT = thrombin clotting time, BT = template bleeding time, LOV = Lovenox, enoxaparin sodium.

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>T+6 hours</th>
<th>Day 2</th>
<th>Day 6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>aPTT (sec)</strong></td>
<td>CONTROL</td>
<td>31.1 ± 1</td>
<td>39.6 ± 2</td>
<td>46.4 ± 5</td>
</tr>
<tr>
<td></td>
<td>PSI-697</td>
<td>29.2 ± 2</td>
<td>37.0 ± 2</td>
<td>39.7 ± 5</td>
</tr>
<tr>
<td></td>
<td>LOV</td>
<td>38.5 ± 3**</td>
<td>46.4 ± 8</td>
<td>47.6 ± 5</td>
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<tr>
<td><strong>TCT (sec)</strong></td>
<td>CONTROL</td>
<td>17.2 ± 1</td>
<td>16.9 ± 0.7</td>
<td>23.4 ± 2</td>
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<tr>
<td></td>
<td>PSI-697</td>
<td>18.6 ± 2</td>
<td>16.6 ± 1</td>
<td>20.7 ± 1</td>
</tr>
<tr>
<td></td>
<td>LOV</td>
<td>22.4 ± 0.3**</td>
<td>21.2 ± 2</td>
<td>39.6 ± 2*</td>
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<tr>
<td><strong>BT (min)</strong></td>
<td>CONTROL</td>
<td>2.8</td>
<td>3.7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>PSI-697</td>
<td>2.3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>LOV</td>
<td>2.5</td>
<td>2.5</td>
<td>3.8</td>
</tr>
<tr>
<td><strong>D-dimer (µg/ml)</strong></td>
<td>CONTROL</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>PSI-697</td>
<td>0 ± 0</td>
<td>0.6 ± 0*</td>
<td>1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>LOV</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0.8 ± 0.8</td>
</tr>
</tbody>
</table>

Elevated D-dimer levels are defined as ≥0.20 µg/ml.
demonstrating this similarity to humans (43). Also, the coagulation system of baboons closely resembles humans (44). We have found the baboon to be an extremely useful and predictive model for the study of the pathophysiology and treatment of DVT.

Stimulation of the venous endothelium initiates P-selectin:PSGL-1 ligand interactions which promote the tethering of neutrophils and activated platelets onto the stimulated vascular endothelium. These events also facilitate the creation of leukocyte complexes on the vascular surface and in the circulation (45). The use of a recombinant P-selectin ligand antagonist has been shown to decrease venous thrombosis in rodent, feline, and primate models of venous thrombosis in a dose-dependent fashion (15, 19). In a study using this same recombinant P-selectin receptor antagonist to treat two-day-old venous thrombi in baboons, lumen opening was significantly improved without bleeding complications. Based on the results from the above mentioned studies, the use of P-selectin antagonism is a logical therapeutic approach to decrease venous thrombosis. In this study, we have documented the therapeutic effects of an orally active, small-molecule inhibitor of P-selectin in a baboon model of venous thrombosis.

Specifically, PSI-967 significantly increased vein lumen opening at days 2 and 6 despite complete occlusion of the veins at the time of balloon deflation while decreasing vein wall inflammation. These findings are consistent with the results of previous studies that suggest P-selectin inhibition can enhance both endogenous and exogenous fibrinolysis (18, 46, 47) by reducing the prothrombotic accumulation of leukocytes, platelets and tissue factor rich/PSGL-1 containing microparticles on the surface of existing thrombi (45, 48). Of interest, this study identified both an effective plasma concentration of PSI-697 (>400 ng/ml) and a non-effective plasma concentration of the compound (<190 ng/ml) in a baboon model of venous thrombosis. However, it is not known if a higher dose and plasma concentration would completely inhibit clot formation. These data will enable the determination of an effective therapeutic concentration needed for thrombus resolution and inhibition in future animal studies. LOV animals did not form thrombus which was consistent with the treatment anti-coagulant dose of LMWH that was used in this study. It was interesting in the LOV-treated animals that from day 2 to day 6 there was a slight decrease in the overall vein opening compared to the opening measured at the time of balloon deflation. PSI-697 therapy in this animal model did not produce any adverse bleeding complications, as determined by coagulation testing and by clinical observation. Conversely, animals on LMWH therapy had significantly elevated aPTT and TCT times, thus confirming a bleeding potential. Importantly, no clinical differences in circulating platelet levels were noted between the groups.

The D-dimer test is a semi-quantitative indication of fibrinolysis (the breakdown of formed clot). In this study, only animals receiving PSI-697 had elevated circulating D-dimer levels at the 6-h time point post thrombus induction. This suggests the mechanism by with PSI-697 promotes thrombolysis through the activation of the fibrinolytic system. However, at day 2 and day 6, elevated D-dimer levels were noted in all control and PSI-697 animals and levels were higher than in the LOV group. Although not elevated in every LOV animal, the elevated D-dimer levels that were found in non-thrombosed LOV animals indicate that other mechanisms than ongoing thrombolysis may be responsible for the elevated levels at the later time points in these LOV treated animals. These findings may also relate to the decreased sensitivity and specificity of the latex based semi-quantitative D-dimer test used in this study compared to enzyme-linked immunosorbent assays (ELISA) (42, 49–51).

Inflammation was assessed in this study using magnetic resonance venography with Gd-enhancement. Gd is a heavy metal chelate that prolongs the T1 relaxation time of blood, and extravasates selectively into areas with capillary leakage and as such is a non-invasive maker of inflammation. Primates receiving either PSI-697 or LOV had significantly decreased vein wall inflammation by magnetic resonance venography versus controls at day 6 post balloon occlusion. In this study, we documented a decrease in circulating microparticles populations in animals receiving PSI-697 post balloon occlusion. With PSI-697 therapy, this decrease in microparticles was most notable in microparticle populations from leukocyte origin after group comparisons were made (Table 2). Previously we have shown that leukocyte-derived microparticles are associated with venous thrombosis and can be modulated by using an antibody against the P-selectin ligand (P-selectin glycoprotein ligand-1) (52).

In conclusion, this study has shown the effectiveness of an oral inhibitor of P-selectin in increasing vein lumen opening by modifying thrombogenesis and its resolution. It also decreased inflammation in a non-human primate model, complementing recent rodent data (39), without anticoagulation and without any evidence of PE. Importantly, this improvement was achieved without the anti-coagulant effects noted with treatment LMWH. PSI-697 effectively modulates the procoagulant activity initiated by P-selectin:PSGL-1 ligand interaction during thrombogenesis. Lastly, the oral formulation provides potentially a distinct advantage in the clinical setting over s.c. and i.v. administration.

**Abbreviations**

PSI-697, small-molecule inhibitor of P-selectin; LMWH, low-molecular-weight heparin; LOV, Lovenox® (Enoxaparin sodium injection); PSGL-1, P-selectin glycoprotein ligand-1; DVT, deep venous thrombosis; PE, pulmonary embolism; IVC, inferior vena cava; MRV, magnetic resonance venography; TOF, time of flight; Gd = gadolinium enhancement; ROI, region of interest; ACD, acid citrate dextrose; PPP, platelet-poor plasma.
References