Intravenous and Oral Antithrombotic Efficacy of the Novel Platelet GPIIb/IIIa Antagonist Roxifiban (DMP745) and Its Free Acid Form, XV459

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Abstract—Currently used antiplatelet drugs, including aspirin, ticlopidine, and others, are effective against certain but not all of the many endogenous platelet activators. Because of their limited efficacy, a significant number of serious thromboembolic complications still occur, highlighting the need for a more effective therapy. DMP745 (roxifiban), a prodrug of XV459, is a recently discovered, potent antiplatelet agent with high affinity and specificity for platelet GPIIb/IIIa receptors that blocks platelet aggregate formation regardless of the agonist (IC₅₀=0.030 to 0.05 µmol/L) or anticoagulant used for blood collection. DMP745 rapidly converts to its active free-acid form, XV459, which has a comparable high affinity for both resting and activated platelets (Kᵣ=1 to 2 nmol/L) and a relatively slow rate of dissociation from resting platelets. The present study was undertaken to determine intravenous and oral antithrombotic efficacies of DMP745 and XV459 and to compare them with those of other antiplatelet and anticoagulant agents in canine models of arterial thrombosis. In these models, thrombosis was induced either electrolytically (200-µA anodal current) in the carotid artery or mechanically by external clamping of the femoral artery along with stenosis, which resulted in either total occlusive thrombus formation or cyclic flow reduction, respectively. DMP745 and XV459 were given either intravenously (0.1 mg/kg bolus) or orally (0.1 to 0.4 mg/kg). Additionally, the antithrombotic efficacies of DMP745, aspirin, heparin, and ticlopidine in the canine carotid artery electrolytic injury model were compared. DMP745 demonstrated oral bioavailability of 20.8% in dogs after administration at different doses and prevented cyclic flow reduction (ED₉₀–₁₀₀= <0.1 mg/kg IV or PO). Additionally, both DMP745 and XV459 (0.1 mg/kg IV or 0.3 to 0.4 mg/kg PO) demonstrated maximal antithrombotic efficacy in preventing electrically induced carotid and coronary artery thrombosis and significant antithrombotic efficacy (P<0.001) at relatively low doses in different settings of arterial thrombosis in the canine model. DMP745 resulted in a significant reduction in thrombus mass and sustained arterial blood flow with 100% prevention of occlusive and nonocclusive thrombosis. In contrast, administration of aspirin (10 mg/kg PO for 2 days), heparin (10 IU/kg IV bolus followed by 90 IU/kg IV infusion over 3 hours), or ticlopidine (300 mg/kg PO for 3 days) before initiation of arterial thrombosis did not reduce the incidence of electrolytic injury–induced occlusive arterial thrombosis. These studies demonstrated a distinct antithrombotic efficacy of DMP745 as compared with existing strategies and suggest potential intravenous and oral antithrombotic uses of DMP745 in the prevention and treatment of thromboembolic disorders. (Arterioscler Thromb Vasc Biol. 1999;19:2535–2541.)

Key Words: platelet GPIIb/IIIa ■ integrin ■ thrombosis ■ antiplatelet ■ anticoagulant ■ roxifiban

Intravascular thrombosis is one of the most frequent pathological events and a major cause of morbidity and mortality in western civilization. Critical steps in the development of acute coronary syndromes are the disruption, rupture, and erosion of atherosclerotic plaque with the formation of an either partially or completely occlusive thrombus.¹–³ Factors that stimulate thrombosis include vascular damage, stimulation of platelets, and activation of the coagulation cascade. Platelet adhesion to exposed subendothelium surfaces of injured vessels with subsequent activation and the resulting aggregation have been shown to be associated with various pathological conditions, including cardiovascular and cerebrovascular thromboembolic disorders, such as unstable angina, myocardial infarction, transient ischemic attack, stroke, and atherosclerosis.³–⁷

The platelet glycoprotein IIb/IIIa complex (GPIIb/IIIa) has been identified as the final common pathway for all platelet agonists.⁸,⁹ The binding of adhesive proteins, such as fibrinogen, to GPIIb/IIIa causes platelets to aggregate.⁹,¹⁰ The binding of fibrinogen is mediated in part by the RGD recognition sequence, which is common to the adhesive proteins that bind to GPIIb/IIIa receptors.⁸–¹⁰ The first platelet GPIIb/IIIa antagonist developed was the chimeric 7E3 (ReoPro).¹¹ ReoPro effectively inhibits platelet aggregation against all known platelet agonists in experimental animals
and humans.12,13 However, the use of monoclonal antibodies as therapeutic agents might present certain limitations, such as immunogenicity, lack of oral bioavailability, and reversibility after intravenous administration. Thus, many groups have concentrated on developing small-molecule GPIb/IIa antagonists.14–19 The approach taken by the different groups has been to develop analogs of RGD or modifications of the RGD sequence to improve the pharmacodynamic (affinity and specificity for platelet GPIb/IIa integrin receptors) and the pharmacokinetic properties of the analogs.16–19

In contrast to the platelet GPIb/IIa antagonist approach, current antiplatelet drugs, including aspirin, ticlopidine, thromboxane A2 synthetase inhibitors, and hirudin, are mainly effective against 1 of the many platelet activators. Hence, the potential clinical benefits of an agent that inhibits platelet aggregation in response to all of these agonists should represent a more efficacious therapeutic approach than provided by current platelet inhibitors.4–6,18 Additionally, a higher incidence of coronary artery reocclusion after successful thrombolytic therapy or percutaneous coronary intervention is a persistent clinical problem despite the use of aspirin and/or heparin.5,7,19 Thus, prevention of reocclusion with an adjunctive pharmacological agent is being actively pursued with different compounds, including anticoagulant and antiplatelet agents. Previous reports have described the clinical implications of different intravenous platelet GPIb/IIa antagonists, such as c7E3, Integrelin, tirofiban (Aggrastat), and roxifiban (DMP754), which is the methyl ester prodrug of XV459, the free-acid active form of XV459, which is methyl N\[^{2}-(3-(4-formamidinophenyl)-isoxazolin-5(R)-yl))-acetamido]-N\[^{1}-(3-carboxy-2,3-\{S\}-diaminopropionate. XV459 is the free-acid active form of DMP754, which is \(N\[^{2}\)-[3-(4-formamidinophenyl-isoxazolin-5(R)-yl)]-acetyl\]-N\[^{1}\)-[1-butyloxycarbonyl]-2,3-(S)-diaminopropionate.

RT6000 tabletop centrifuge with H-1000 B rotor (DuPont) at room temperature, and platelet-rich plasma (PRP) was removed. The remaining blood was centrifuged for 10 minutes at 1500g at room temperature, and platelet-poor plasma (PPP) was removed. Samples were assayed on a PAP-4 platelet profiler using PPP as the blank (100% transmittance). Twenty microliters of the platelet agonist, ADP (final concentration, 100 \(\mu\)mol/L), was then added to each tube, and aggregation profiles were plotted (% transmittance versus time). Maximal aggregation was obtained with ADP at a final concentration of 100 \(\mu\)mol/L. Twenty microliters of XV459 was added at different concentrations before the addition of ADP (100 \(\mu\)mol/L). Results are expressed as the percentage of inhibition of agonist-induced platelet aggregation or as the \(I_{50}\) (\(\mu\)mol/L).

**Antiplatelet Efficacy in Anesthetized Dogs**

Mongrel dogs of either sex were anesthetized by administration of Nembutal sodium solution (30 mg/kg IV, Abbott Laboratories). Animal studies were approved by the Animal Care and Use Committee of DuPont Pharmaceuticals. DMP754 was administered orally (single dose) in soft gelatin capsules at a dose of 0.1 or 0.3 mg/kg or intravenously as a 0.1-mg/kg bolus. XV459 was administered orally (single dose) in soft gelatin capsules at a dose of 0.4 mg/kg or intravenously at a dose of 0.1 mg/kg. Blood samples were placed on a platform rocker until assayed for platelet aggregation within 2 hours as previously described. The antiplatelet efficacy of DMP754 and XV459 was calculated by comparing the percentage of aggregation in samples after administration of the agents to percentages in baseline samples from the same animal.

**Arterial Thrombosis Model in Dogs**

In these models, different subsets of arterial thrombosis were induced either (1) electrolytically (200- \(\mu\)A anodal current) in the right or left carotid artery (CA) (occlusive thrombosis) or (2) mechanically by external clamping of the right or left femoral artery (FA) (4 to 5 times) along with stenosis (cyclic flow reduction [CFR]). Each animal served as its own control for the intravenous route of administration. To study oral dosing, DMP754 or XV459 was administered 30 to 60 minutes before anesthesia to minimize the effect of the anesthetic on oral absorption of the test agent. DMP754 or XV459 was administered intravenously or orally, and the same protocol of induction of arterial thrombosis was repeated and extended for 3 hours. Arterial flow, ex vivo platelet aggregation and template bleeding time, incidence of occlusion, time to occlusion, incidence of CFR, and thrombus weight were monitored throughout the procedure.

**FA Thrombosis in Dogs (Folts’ Model)**

Mongrel dogs of either sex weighing 8 to 15 kg were anesthetized and handled as previously described. The left and right femoral artery (FA) was dissected and freed from fascia and branches for a distance of 15 to 20 mm. A Doppler flow probe was placed around...
Arterial Thrombosis Model in Dogs

Femoral Artery - Mechanical Injury + Stenosis

Control Side

Treated Side

Figure 2. Representative tracing showing the antithrombotic efficacy of DMP754 (0.3 mg/kg PO) in blocking CFR in canine FA thrombosis model.

the distal portion of the vessel segment, and flow was monitored throughout the study. Animals were allowed to stabilize for 20 minutes, and the hyperemic response of the dissected FA was determined by 2 repeated, brief (20-second) total occlusions (3 to 5 minutes apart). After restoration of basal flow for 20 minutes, a 2.5-mm-long plastic cylinder was placed on the proximal portion of the FA, creating a critical stenosis that reduced the lumen area of the vessel by up to 80%, thereby preventing the hyperemic response while minimally affecting basal flow. The clip was then moved onto one side, and a portion of the FA was mechanically damaged by gentle clamping. The stenotic clip was then moved back onto the damaged segment of the vessel. This resulted in repeated CFRs followed by restoration of flow on dislodging or gentle shaking around the clip. Baseline measurements of the frequency of CFRs were obtained, and then either saline (control group) or DMP754 or XV459 (treated groups) was administered intravenously as a single bolus (0.1 mg/kg) or orally (0.1 and 0.3 mg/kg). CFRs were monitored for up to 3 hours after treatment. Figure 2 is a representative tracing showing the antithrombotic efficacy (100% prevention of CFR) of DMP754 at 0.3 mg/kg PO.

CA Thrombosis in Dogs (Electrolytic Injury Model)

The experimental procedure results in formation of a platelet-rich intravascular thrombus at the site of the electrolytically induced lesion. The response of the CA to the electrolytic injury is similar to that observed in the canine coronary artery, in which intimal wall injury secondary to application of a direct anodal current leads to injury secondary to application of a direct anodal current leads to thrombus formation. Animals were instrumented as described earlier. A 20- to 30-mm segment of the left or right CA was exposed and freed from fascia and branches were tied. Anodal current was applied using an intravascular electrode composed of a Teflon-insulated, silver-coated copper wire (28 gauge). Penetration of the vessel wall by the electrode was facilitated by attaching the tip of a 23-gauge hypodermic needle to the uninsulated part of the electrode. Each intraarterial electrode was connected to the positive pole (anodal) of a dual-channel stimulator (Ni-Cad battery, 9 volts, connected to 250 000-Ohm potentiometer in series). The cathode was connected to a distant subcutaneous site. The current delivered to the arterial wall was monitored continuously and maintained at 200 μA. Proper positioning of the electrode in the CA was confirmed by visual inspection at the end of each experiment. In all experiments, the anodal current was applied for a maximum of 3 hours. Flow was monitored throughout the experiment by use of a Doppler flow probe (model 100, Triton Technology). Figure 3 is a representative tracing showing the antithrombotic efficacy (100% prevention of occlusive thrombus formation) of DMP754 at a dose of 0.3 mg/kg PO.

Pharmacokinetics

Animals were fasted overnight and received standard certified commercial dog food (400 g, Wayne Certified Dog Chow no. 8727) 6 hours after administration of the dose over a 2-hour feeding period and were given water ad libitum. Four dogs received 0.04 mg/kg XV459 in 5% dextrose in water for injection as a single intravenous dose via the jugular vein, and 4 dogs received 0.40 mg/kg DMP754 in distilled water as a single oral gavage dose by means of intubation. Blood samples (1.2 mL) were collected from the jugular vein by venipuncture at predetermined time points into heparinized Vacutainers (Becton-Dickinson) for plasma measurements of XV459.

Arterial Thrombosis Model in Dogs

Coronary Artery - Electrolytic Injury + Stenosis

Control Side

Treated Side

Time to occlusion: 51 ± 5 min. (n = 20)

Time to occlusion: >160 min.

Figure 3. Representative tracing showing the antithrombotic efficacy of DMP754 (0.3 mg/kg PO) in blocking occlusive thrombosis in canine CA thrombosis model.

Liquid Chromatography/Mass Spectrometry/Mass Spectrometry Assay for XV459

A specific and sensitive assay for the determination of XV459 in dog plasma was developed using liquid chromatography/mass spectrometry via a turbo ion spray ionization source. The method involved solid-phase extraction of XV459 and the internal standard (XU066) from plasma using a C-8 end-capped column. The mobile phase consisted of acetonitrile, water, and 98% formic acid (20:80:0.1, vol/vol/vol) running in an isocratic mode. The analytes were separated on a 1-mm×150-mm YMC basic S5 micobore column at a flow rate of 150 μL/min. The analytes eluting from the column were ionized in the turbo ion spray ionization source and sampled into the Sciex API III+ triple quadrupole mass spectrometer. The mass spectrometer was operated in multiple-reaction monitoring mode, in which parent ion (with a mass/charge ratio [m/z] of 434 [XV459]), internal standard (XU066) (with a m/z of 448), and their fragment ions (with m/z values of 212 and 133, respectively) were monitored.

Statistical Analysis

Data are expressed as mean±SEM. The design of the experimental protocol in certain sections of the study allowed each animal to serve as its own control for baseline values (% aggregation). Data were analyzed by either paired or group analysis using Student’s t test or ANOVA when applicable; differences were considered significant at P<0.05.

Results

In Vitro Antiplatelet Efficacy

DMP754 administered after treatment with liver esterases and its free-acid form inhibited, in a concentration-dependent manner, agonist-induced (100 μmol/L ADP) platelet aggregation in platelets obtained from either dogs or humans (IC50 = 0.024 to 0.032 μmol/L) (Table 1).

Antiplatelet Efficacy in Dogs

Ex Vivo Platelet Aggregation

DMP754 demonstrated maximal antiplatelet efficacy when administered at a dose of 0.1 mg/kg IV bolus or 0.3 mg/kg PO

TABLE 1. In Vitro Antiplatelet Effects of DMP754 or Its Free Acid Form XV459 in Human and Dog PRP

<table>
<thead>
<tr>
<th>Species</th>
<th>DMP754†</th>
<th>XV459</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>0.032</td>
<td>0.024</td>
</tr>
<tr>
<td>Mongrel dog</td>
<td>0.025</td>
<td>0.027</td>
</tr>
</tbody>
</table>

†DMP754 was treated with liver esterases for 2 h at 37°C.

Platelet aggregation (light transmittance aggregometry) was induced by adding ADP at 100 μmol/L to PRP.

Values are mean IC50 (n=3–12).

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in anesthetized dogs (Table 2). In contrast, orally administered DMP754 at a dose of 0.1 mg/kg showed much lesser antiplatelet efficacy (Table 2). Additionally, maximal antiplatelet efficacy was demonstrated with XV459 at a dose of 0.1 mg/kg IV (Table 2). Similarly, XV459 demonstrated maximal antiplatelet efficacy at 0.4 mg/kg PO (Table 2). Neither DMP754 nor XV459 affected platelet counts in whole blood (data not shown).

**Bleeding Time**
Both DMP754 and XV459 at a dose of 1.0 mg/kg IV significantly (P<0.001) extended bleeding time to >30 minutes, as compared with a baseline bleeding time of 3 to 4 minutes. Platelet bleeding time increased to 6 to 8 minutes after administration of 0.1 mg/kg PO DMP754, extended to 12 to 15 minutes after administration of 0.4 mg/kg PO of XV459, and extended to 15 to 20 minutes after administration of 0.3 mg/kg PO of DMP754.

**Pharmacokinetic Parameters**
The pharmacokinetics of XV459 after intravenous administration and its bioavailability after oral administration of DMP754 were investigated in dogs. After administration of single intravenous bolus doses of 0.04, 0.4, and 1.0 mg/kg of XV459 in dogs, plasma concentrations of XV459 declined polyexponentially. The terminal half-life (t1/2) was comparable for all 3 doses, with mean values of 10.4, 11.8, and 12.2 hours, respectively. However, the systemic plasma clearance (CL) and volume of distribution at steady state (Vss) of XV459 increased with dose. The mean CL values were 1.0, 4.1, and 6.3 mL/min per kg, whereas the mean Vss values were 0.8, 3.4, and 4.4 L/kg at doses of 0.04, 0.4, and 1.0 mg/kg, respectively.

The gastrointestinal absorption rate of DMP754 from normal saline was moderate, with peak plasma concentrations of XV459 attained within 1.4 hours after oral administration of 0.4 mg/kg DMP754 in aqueous solution. Plasma concentrations of XV459 had a mean Cmax of 93.9 ng/mL. The apparent bioavailability (F), defined as the percentage of XV459 formed after oral administration of DMP754, was 20.8% (Table 3). The postabsorptive plasma concentrations of XV459 declined monoexponentially as a function of time.

**Distribution of XV459 Between Platelet and Plasma Compartments**
DMP728, a potent platelet GPIIb/IIIa antagonist with a relatively fast rate of dissociation from platelets, was used in the present study to compare its platelet/plasma distribution to that of XV459. The distribution of 3H-XV459 in human blood was compared with that of 3H-DMP728. Total blood XV459 concentration (dissociated from platelets by EDTA plus free in plasma) was markedly greater (10- to 12-fold) than corresponding free plasma concentrations (Table 4). In contrast, 3H-DMP728 demonstrated equilibrium between PRP and PPP (Table 4).

**Antithrombotic Efficacy in Dogs**

**Femoral Artery (FA) Thrombosis in Dogs (Folts’ Model)**
In this model, a platelet-dependent thrombus is produced in a mechanically injured FA in the presence of a high degree of arterial constriction. Under these conditions, FA blood flow is occluded as a platelet-rich thrombus forms, which is followed by restoration of flow on dislodging or gentle shaking around the clip; this process results in thrombus formation and disappearance in a cyclic manner (CFRs). This study exam-
ined the antithrombotic efficacy of DMP754 and XV459 at doses of 0.1 mg/kg IV or PO and 0.3 to 0.4 mg/kg PO. DMP754 and XV459 both demonstrated maximal antithrombotic efficacy at 0.1 mg/kg IV and at 0.3 to 0.4 mg/kg PO in anesthetized dogs (Table 5). At these doses, DMP754 and XV459 significantly prevented the incidence of CFR during the 3 hours of the study (Table 5). In contrast, both aspirin and ticlopidine were effective only in reducing, not preventing, CFR in 30% to 40% of animals (data not shown).

**CA Thrombosis in Dogs (Electrolytic Injury Model)**

The effect of DMP754 and XV459 on the incidence of occlusion, time to occlusion, and thrombus weight of an electrotyically induced thrombus in a canine CA model was examined. DMP754 administered at doses of 0.1 mg/kg IV and 0.3 to 0.4 mg/kg PO before the insult resulted in 100% prevention of the incidence of occlusion (Table 5). Additionally, both DMP754 and XV459 resulted in significant prolongation of the time to occlusion along with a significant reduction ($P<0.01$) in the weight of the thrombus formed (Table 5). A significant prolongation of the time to occlusion to $>180$ minutes (ie, 100% prevention of the incidence of occlusion for up to the maximum period of the study) was demonstrated. In contrast, aspirin (10 mg/kg PO for 2 days), heparin, and ticlopidine (300 mg/kg PO for 3 days) administered before initiation of arterial thrombosis did not reduce the incidence of occlusive arterial thrombosis (Table 6).

**Discussion**

New targets for antiplatelet therapy have been identified as a result of a better understanding of the different processes involved in arterial thrombosis.18,30 Once endothelial damage occurs, platelet thrombus formation advances in 3 steps, including firstly, platelet adhesion and secondly, platelet activation by the various agonists or activators from damaged endothelium and from within activated platelets. The third and final step in platelet rich-thrombus formation is mediated exclusively by the GPIIb/IIIa receptor. Various large-scale, phase III clinical trials have illustrated the usefulness of ReoPro in the treatment of percutaneous coronary interventions.27,30 The first such study was the pivotal Evaluation of Ilb/IIIa Platelet Receptor Antagonists 7E3 in Preventing Ischemic Complications (EPIC) trial, which enrolled >2000 high-risk patients scheduled to undergo percutaneous intervention. Although investigators in the EPIC trial found a relative 35% (13.1% versus 7.7%, $P=0.008$) reduction in the rate of composite end-point events in patients treated with ReoPro at 30 days, they also raised concern about excess and significant bleeding complications.13 In the Evaluation in

### TABLE 4. Human PRP/Plasma Ratios of XV459 and DMP728

<table>
<thead>
<tr>
<th>Compound</th>
<th>Platelets/Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>XV459</td>
<td>10/1</td>
</tr>
<tr>
<td>DMP728</td>
<td>1/1</td>
</tr>
</tbody>
</table>

All compounds were $^3$H-labeled. EDTA at 10 mmol/L resulted in dissociation of platelet-bound radiolabel GPIIb/IIIa antagonists. XV459 binds with affinity to and has slow dissociation from resting human platelets compared with DMP728. DMP728 is a potent GPIIb/IIIa antagonist with a relatively fast dissociation from resting platelets.

### TABLE 5. Antithrombotic Efficacy of Intravenous and Oral DMP754 and XV459 in Canine Thrombosis Model

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time to CFR, h</th>
<th>Time to Occlusion, min</th>
<th>CBF, cm/sec</th>
<th>Thrombus Weight, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>8±5</td>
<td>88.6±3.7</td>
<td>25.2±1.2</td>
<td>46.3±2.7</td>
</tr>
<tr>
<td>XV459</td>
<td>0±0</td>
<td>&gt;180</td>
<td>20.0±2.0</td>
<td>17.3±4.6</td>
</tr>
<tr>
<td>DMP754</td>
<td>0±0</td>
<td>&gt;180</td>
<td>24.7±5.4</td>
<td>11.3±1.3</td>
</tr>
</tbody>
</table>

Values are mean±SD (n=4–6 per group). CA indicates carotid artery; CBF, carotid blood flow.

*Time to occlusion versus incidence of occlusion: All control animals totally occluded by the time stated above (i.e., incidence of occlusion=100%). All treated animals with time to occlusion $>180$ min, the incidence of occlusion was 0%. The time to occlusion did not significantly affected in the group treated with 0.1 mg/kg, PO (ie, incidence of occlusion=100%).

| P<0.05, ‡P<0.001.  |

### TABLE 6. Antithrombotic Efficacy of DMP754 Compared With Other Antiplatelet or Anticoagulant Strategies in Canine Thrombosis Model (CA-Electrolytic Occlusive Thrombosis)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time to Occlusion, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>80±4</td>
</tr>
<tr>
<td>DMP754</td>
<td>&gt;180*</td>
</tr>
<tr>
<td>Aspirin</td>
<td>92±8</td>
</tr>
<tr>
<td>Heparin</td>
<td>95±10</td>
</tr>
<tr>
<td>Ticlopidine</td>
<td>110±12</td>
</tr>
</tbody>
</table>

Values are mean±SD (n=5–6 per group). DMP754 (0.3 mg/kg PO), aspirin (10 mg/kg PO for 2 d), heparin (10 IU/kg IV bolus followed by 90 IU/kg IV infusion over 3 h), and ticlopidine (300 mg/kg PO for 3 d) were administered before initiation of arterial thrombosis.

*P<0.001.
PTCA to improve long-term outcome with Abciximab GpIIb/IIIa blockade EPILOG trial, a decrease in the incidence of bleeding was documented with lowering of the heparin dose. Additionally, a small-molecule, nonpeptide GPIIb/IIIa antagonist, tirofiban (Aggrastat) had significant clinical benefits in patients with unstable angina and non-Q-wave myocardial infarction (PRISM and PRISM-PLUS trials).³

Several other selective GPIIb/IIIa antagonists, including Integrelin, tirofiban (Aggrastat), and lamifiban, are in advanced stages of clinical development and are being developed primarily for intravenous use in the treatment and prevention of acute ischemic heart diseases. The Integrelin to Manage Platelet Aggregation to Prevent Coronary Thrombosis (IMPACT II) and Platelet glycoprotein IIb/IIIa in Unstable angina: Receptor Suppression Using Integrin Therapy (PURSUIT) trials with Integrelin and the Randomized Efficacy Study of Tirofiban for Outcomes and ReStenosis (RESTORE) trial with tirofiban (Aggrastat), as well as other trials, have demonstrated significant clinical benefits in acute ischemic syndromes. Clinical studies with orally active GPIIb/IIIa antagonists, including xemilofiban (SC54684) and lefradafiban (BIBU104), demonstrated oral antiplatelet activity in humans with administration 2 to 3 times per day.²⁵,²⁶

The active free-acid form of Roxifiban has distinct platelet GPIIb/IIIa-binding characteristics along with a potent in vitro antiplatelet efficacy regardless of the activator or anticoagulant used for blood collection (citrate versus heparin).²⁷,²⁹ Those attributes result in the unique pharmacodynamic and pharmacokinetic properties of roxifiban shown in the present study. XV459 has been demonstrated to have high affinity for platelet GPIIb/IIIa and similar potency in inhibiting platelet aggregation regardless of the agonist or anticoagulant used.²⁸,²⁹ XV459 inhibited platelet aggregation and had comparable IC₅₀ values regardless of the concentration of ADP used (10 or 100 μmol/L).²⁹ XV459 has been shown to be a competitive inhibitor with high affinity for inhibiting fibrinogen binding to platelet GPIIb/IIIa receptors.²⁹ A comparable high-affinity binding (Kᵢ=0.0008 to 0.0025 μmol/L) of radiolabeled XV459 to either activated or unactivated platelets obtained from humans, baboons, or dogs was also demonstrated.²⁷,²⁹ XV459 demonstrated a high degree of selectivity toward the platelet GPIIb/IIIa receptors as compared with the closely related vitronectin receptors on endothelial cells or other adhesion receptors.²⁸ Additionally, XV459 demonstrated high affinity for both activated and unactivated platelets along with relatively slow dissociation rates, suggesting a possible prolonged duration of in vivo antiplatelet effects.²⁹ L-738,167, a nonpeptide GPIIb/IIIa antagonist, has been evaluated in various animal models and has been found to have an extended duration of antiplatelet efficacy and a slow rate of dissociation from platelets.³²,³³ This is in contrast to current intravenous platelet GPIIb/IIIa antagonists, such as Integrelin, tirofiban (Aggrastat), or lamifiban, and DMP728, which have a short duration of antiplatelet effects associated with their relatively fast rates of dissociation from human platelets.¹⁸,²⁷ Because platelet glycoprotein GPIIb/IIIa receptor–specific antagonists, in addition to binding to plasma proteins, are also bound to blood platelets, an appreciable quantity of a compound in the platelet-rich buffy coat after conventional centrifugation of citrated blood might be lost. Therefore, drug concentrations in citrated PPP samples could be substantially lower than those in citrated PRP. EDTA, in addition to its anticoagulant property, may be used as a potential releaser of the drug bound to platelets by dissociating the calcium-dependent GPIIb/IIIa heterodimer. Hence, the type of biological matrix used for quantitation of drug concentrations at therapeutic levels could play a major role in the estimation of pharmacokinetic and pharmacodynamic parameters.³⁴ Results demonstrated that XV459 exhibited nonlinear, dose-dependent pharmacokinetics in beagle dogs. The nonlinearity in pharmacokinetics suggested that prediction of plasma concentrations of XV459 after a certain dose and time will be difficult. The in vitro plasma protein-binding values of XV459 in fresh dog PRP at 5, 25, and 100 ng/mL were 85%, 85%, and 59%, respectively. The nonlinear clearance and distribution characteristics of XV459 appeared to be related to the saturable binding of XV459 to platelets.

Antiplatelet efficacy of DMP754 and XV459 was shown for the doses used in the antithrombotic efficacy studies. DMP754 has minimal antiplatelet efficacy when given at a dose of 0.1 mg/kg PO and maximal antiplatelet efficacy at a dose of 0.3 mg/kg PO. This again illustrates the steep dose-response relation of this class of compounds. DMP754 at 0.3 mg/kg PO is as effective as 0.4 mg/kg PO of XV459, suggesting improved oral antiplatelet efficacy of the produg form (DMP754). This was further demonstrated by the improved antithrombotic efficacy of DMP754 (0.3 mg/kg PO) as compared with XV459 (0.4 mg/kg PO) in limiting thrombus growth and improved CA blood flow. A potent oral antithrombotic effect of DMP754 was shown at relatively low unit doses (0.3 to 0.4 mg/kg PO) as compared with the relatively higher unit dose required with other orally active GPIIb/IIIa antagonists, such as SC-54684A (2.5 to 5.0 mg/kg PO BID in dogs).²³ In the CA thrombosis model in dogs, DMP754 (0.1 to 0.3 mg/kg IV or PO) exhibited antithrombotic efficacy in electrolytically induced CA thrombosis. DMP754 at a dose of 0.3 mg/kg PO is more effective than XV459 at a dose of 0.4 mg/kg PO in limiting thrombus weight and blood flow in electrolytic injury–mediated occlusive thrombosis in the CA (Table 3). In the case of increased thrombogenic stimulus mediated by electrolytically induced arterial thrombosis in the CA, DMP754 was capable of limiting thrombus formation at the site of damage. However, it remains to be determined in a clinical setting how long after thrombolysis platelet inhibition must be sustained to render the injured vessel and residual thrombus mass nonthrombogenic. In contrast, aspirin (10 mg/kg PO for 2 days) and ticlodipine (300 mg/kg PO for 3 days) administered before initiation of arterial thrombosis were effective in reducing the incidence of CFR but were ineffective in reducing the incidence of electrolytic injury–induced occlusive arterial thrombosis. Additionally, in the same models, hirudin, but not heparin, was shown to have antithrombotic efficacy, which was reversed on rechallenge with epinephrine (data not shown). The improved antithrombotic efficacy of platelet GPIIb/IIIa antagonists as compared with other agents might be due the universal effectiveness against all known platelet activators.³⁵ It is clear that the efficacious doses vary according to the type of models used. In the Folts’ model, in which the thrombus is mainly platelet-dependent, relatively lower oral doses of DMP754 (0.1 mg/kg) resulted in maximal
antithrombotic efficacy. In contrast, in the electrolytic injury model, in which the thrombus is associated with mixed platelet, fibrin, monocyte, and red blood cells, relatively higher doses of DMP754 (0.3 mg/kg PO) are required for maximal antithrombotic efficacy. These data suggest that DMP754, a low-molecular-weight GPIIb/IIIa receptor antagonist, may have therapeutic potential as an effective intravenous and oral antithrombotic agent in the prevention and treatment of thromboembolic disorders.

References
Intravenous and Oral Antithrombotic Efficacy of the Novel Platelet GPIIb/IIIa Antagonist Roxifiban (DMP754) and Its Free Acid Form, XV459
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