Antiplatelet Efficacy of XV459, A Novel Nonpeptide Platelet GPIIb/IIIa Antagonist: Comparative Platelet Binding Profiles with c7E3

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ABSTRACT
Recent advances in the development of i.v. platelet glycoprotein IIb/IIIa antagonists led to the development of either a class of small-molecular-weight antagonists with a short to ultra-short duration of antiplatelet effects (Integrelin, Tirofibran, DMP728) or a very long-acting antagonist (ReoPro). Thus the present study was undertaken to characterize the antiplatelet efficacy of a small-molecule GPIIb/IIIa antagonist, DMP754/XV459, and to determine its platelet GPIIb/IIIa receptor binding profiles. DMP754, upon its conversion with arginylglycylaspartic acid (RGD) or a very long-acting agonist peptide (10 μM), thrombin receptor agonist peptide (10 μM) or collagen (20 μg/ml) in citrate or heparin. Maximal platelet aggregation inhibition was achieved at 50 to ∼80% receptor occupancy, depending on the agonist used. Both XV459 and c7E3 bind with high affinity to either activated human platelets (Kd = 0.0008 and 0.0091 μM, respectively) or unactivated human platelets (Kd = 0.0025 and 0.0092 μM, respectively). XV459 demonstrated tight association with human, baboon and (to a lesser extent) canine platelets (t½ of dissociation = 7 ± 0, 8 ± 1 and 1.4 ± 0.1 minutes, respectively). Both c7E3 and XV459 associate tightly with slower dissociation rates to unactivated human platelets. XV459 represents a potent antiplatelet agent in inhibiting platelet aggregation along with offering high affinity and a relatively slow dissociation rate from human platelet GPIIb/IIIa receptors that might allow for once-a-day p.o. dosage.

Intravascular thrombosis is one of the most frequent pathophysiological processes in the Western world. Critical steps in the development of acute coronary syndromes include the disruption or erosion of atherosclerotic plaque with the formation of partially or completely occlusive thrombi (Davies, 1990; Fuster, 1994). Factors that stimulate thrombosis include vascular damage, stimulation of platelets and activation of the coagulation cascade. Platelet adhesion to exposed subendothelial surfaces of injured vessels, with subsequent activation and the resulting aggregation, has 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 (Ashby et al., 1990; Davies and Thomas, 1985; Fitzgerald et al., 1986; Fuster et al., 1985; Hamm et al., 1987; Rubenstein et al., 1981; Smitherman et al., 1981; Willerson et al., 1989). Injury of blood vessel walls could occur either acutely or chronically during various pathophysiological processes (Ashby et al., 1990; Davies and Thomas, 1985; Willerson et al., 1989). Platelets then adhere to injured vessel wall and are activated by a number of activators or agonists that are released from within platelets or from the injured arterial walls, with the subsequent aggregation and resultant formation of an occlusive thrombus in the lumen of the vessel (Fitzgerald et al., 1986; Fuster et al., 1985; Hamm et al., 1987; Kruithof et al., 1986; Smitherman et al., 1981). A number of agonists, generated at the interface between the vessel wall and circulating blood at the site of vascular injury, have been shown to activate platelets. These include ADP, epinephrine, thromboxane A2 and thrombin in the fluid phase, collagen, and other components of the extracellular matrix in the subendothelium (Ashby et al., 1990; Fitzgerald et al., 1986).

The platelet GPIIb/IIIa, a membrane protein mediating fibrinogen binding, has been identified as the final common pathway for agonist-induced platelet aggregation (D’Souza et al., 1990; Philips et al., 1991; Pytela et al., 1986). GPIIb/IIIa in activated platelets is known to bind four soluble adhesive proteins: fibrinogen, von Willebrand factor (VWF), fibronectin, and proteoglycans. GPIIb/IIIa on the platelet membrane is a heterodimer of GPIIb (a 105-kDa glycoprotein) and GPIIIa (b3 integrin: Arg-Gly-Asp (RGD) binding domain). The GPIIb/IIIa macromolecule clusters on the platelet surface by binding an RGD sequence in fibrinogen and other adhesive proteins, which provide a new surface for interactions with additional adhesive proteins that can also bind to GPIIb/IIIa, such as fibrin and von Willebrand factor.

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ABBREVIATIONS: GPIIb/IIIa, glycoprotein IIb/IIIa integrin; RGD, Arg-Gly-Asp; PRP, platelet-rich plasma; PPP, platelet-poor plasma; TRAP, thrombin receptor agonist peptide; P.O., Oral.
tin and vitronectin. The binding of fibrinogen and VWF to GPIIb/IIIa causes platelets to aggregate. 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. Several RGD-containing peptides have been shown to block fibrinogen binding and to prevent the formation of platelet thrombi (Cook et al., 1994; Mousa et al., 1993; Mousa et al., 1994; Mousa and Bennett, 1996; Nichols et al., 1992; O’Neil et al., 1994). However, their therapeutic utility is limited by the low affinity and/or the lack of oral bioavailability.

Several studies have identified the pivotal role of the platelet GPIIb/IIIa receptor in coronary thrombosis, and this platelet integrin receptor has emerged as a rational therapeutic target in the management of acute coronary syndromes (Coller et al., 1986; Kleiman et al., 1993; Peerlinck et al., 1993; Simoons et al., 1994; Topol and Plow, 1993; Topol, 1995; Tcheng et al., 1995; Tcheng, 1997). Studies in the human with a monoclonal antibody for GPIIb/IIIa (c7E3) have suggested the antithrombotic benefit of GPIIb/IIIa antagonism, a result that is in agreement with the initial preclinical investigation in animal models (Coller et al., 1986; Mousa and Bennett, 1996; Topol and Plow, 1993; Topol, 1995; Yasuda et al., 1990). The i.v. administration of c7E3 Fab antibody (abciximab, ReoPro) in high-risk patients undergoing angioplasty has been shown to reduce the composite incidence of major ischemic events. In other clinical studies, c7E3 demonstrated efficacy when given in combination with thrombolytic therapy and in refractory unstable angina patients before angioplasty (Kleiman et al., 1993; Simoons et al., 1994). Several other selective GPIIb/IIIa antagonists, including Integrelin, Tirofiban (MK383), and Lamifiban (Ro44-9883) are in advanced stages of clinical development, aimed primarily for i.v. use in the treatment and prevention of acute ischemic heart diseases in the settings of angio-plasty, thrombolyis and unstable angina (Mousa and Topol, 1997; Peerlinck et al., 1993; Tcheng et al., 1995; Tcheng, 1997; Topol and Plow, 1993; Topol, 1995). The i.v. GPIIb/IIIa antagonists currently in clinical trials, such as Tirofiban and Integrelin, dissociate move rapidly from human platelets, which reflects their short duration of antiplatelet effects as compared with that of ReoPro (Peerlinck et al., 1993; Tcheng et al., 1995; Topol and Plow, 1993; Topol, 1995).

Recent clinical studies with orally active GPIIb/IIIa antagonists including Xemilofiban (SC54684) and Fradafiban (BIBU104) demonstrated oral antiplatelet activity in the human when they were administered two to three times per day (Kottke-Marchant et al., 1995; Simpfendorfer et al., 1997; Narjes et al., 1995). As previously discussed, several GPIIb/ IIIa receptor antagonists are under development, and initial clinical studies support their potential use in both low- and high-risk patients undergoing coronary angioplasty (Peerlinck et al., 1993; Tcheng et al., 1995; Tcheng, 1997; Topol and Plow, 1993; Topol, 1995). These factors prompted us to develop a potent GPIIb/IIIa antagonist for the treatment of the different thromboembolic disorders.

DMP 754, a methyl ester prodrug, has been shown to be 100% converted into its free acid active form, XV459, upon exposure to esterases (Mousa et al., 1996). XV459, the active form of DMP 754, demonstrated potent antiplatelet efficacy and specificity to the platelet GPIIb/IIIa receptors (Mousa et al., 1996).

The present study was undertaken to characterize the in vitro antiplatelet efficacy of the platelet GPIIb/IIIa antagonist XV459 and to determine its platelet GPIIb/IIIa receptor binding kinetics as compared with c7E3.

Materials and Methods

Reagents. The following drugs and chemicals were used in this study: ADP, collagen, epinephrine and other reagents used but not specifically mentioned (Sigma Chemical Co., St. Louis, MO), arachidonic acid (Nu check prep, Elusian, MN), TRAP (Peninsula Laboratories Inc., Belmont, CA), 125I-fibrinogen (DuPont NEN, Boston, MA), chimeric 7E3 (c7E3) and 125I-labeled 7E3 (Centocor, Malvern, PA). DMP 754 and its free acid form, XV459 (Fig. 1), were synthesized at DuPont Pharmaceuticals Co. (Wilmington, DE). The active free acid form of DMP 754, XV459 was used in all of the in vitro studies described here.

Antiplatelet Efficacy

Light transmittance aggregometry assay. Venous blood was obtained from healthy human donors who were aspirin-free for at least 2 weeks before blood collection or from other species as previously described (Mousa et al., 1993; Mousa et al., 1994). Briefly, blood was collected into either citrate or heparin Vacutainer tubes. The blood was centrifuged for 10 min at 150 × g in a Sorvall RT6000 Tabletop Centrifuge with H-1000 Rotor) at room temperature, and PRP was removed. The remaining blood was centrifuged for 10 min at 2500 rpm at room temperature, and PPP was removed. Samples were assayed on a PAP-4 Platelet Profiler, using PPP as the blank (100% transmittance). Two hundred microliters of PRP (2 × 10^8 platelets/ml) were added to each micro test tube, and transmittance was set to 0%. Twenty microliters of the platelet agonist ADP (100 μM final concentration) was added to each tube, and the aggregation profiles were plotted (percent transmittance vs. time). Maximal aggregation was obtained with ADP at 10 to 100 μM final concentration. Twenty microliters of XV459 or c7E3 was added at different concentrations 8 min before the addition of ADP (100 μM), TEAC (thrombin (0.001 I.U./ml), epinephrine (100 μM), ADP (100 μM) and collagen (20 μg/ml), TRAP (10 μM) and thrombin (0.5 I.U./ml) in the presence of the fibrin polymerization inhibitor GPRP (glycine-proline-arginine-proline) at 100 μM or collagen (20 μg/ml). TEAC is a mixture of platelet agonists containing maximally effective concentrations of different agonists along with a submaximal concentration of thrombin to minimize excessive fibrin formation. Results were expressed as percent inhibition of agonist-induced platelet aggregation or IC50 (μM).

Platelet GPIIb/IIIa receptor occupancy. Human PRP was prepared as previously described. A 0.04 ml aliquot of radiolabeled...
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Platelet binding kinetics

Platelet binding affinity to activated vs. unactivated platelets. This assay was used to determine a compound’s saturable binding to platelets using PRP. Citrated whole blood (5-ml draw, Vacutainer tubes) was collected from healthy, aspirin-free human subjects, canines, baboons or swine and centrifuged for 10 min at 150 × g. The combination of ADP, epinephrine and sodium arachidonate resulted in maximal fibrinogen binding as compared with other agonists (data not shown). The 125I-fibrinogen bound to the activated platelets, by a mixture of ADP, epinephrine and arachidonate at 100 μM each, was separated from the free form by centrifugation and then counted on a gamma counter. Nonspecific binding (due to entrapment of 125I-fibrinogen) either in the presence or absence of the inhibitors was shown (in the absence of agonists) to be in the range of 4 to 6% of total 125I-fibrinogen binding to agonist-activated platelets. Percent inhibition of 125I-fibrinogen binding to activated platelets was calculated by dividing the specific binding in the presence by the percent of the absence. For IC50 determination, XV459 was added at various concentrations prior to platelet activation.

Results

Antiplatelet Efficacy Studies

Platelet aggregation: light transmittance aggregometry. In the human PRP light transmittance assay, XV459 demonstrated high potency (IC50 = 0.030–0.060 μM) in inhibiting human platelet aggregation induced by ADP (100 μM), TRAP (10 μM) or collagen (20 μg/ml) in either citrate or heparin (fig. 2; table 1). A relatively higher potency for XV459 in inhibiting human platelet aggregation induced by ADP (100 μM), thrombin (0.5 I.U./ml) or a mixture of agonists, TEAC (thrombin (0.001 I.U./ml), epinephrine (100 μM), ADP (100 μM) and collagen (20 μg/ml)) was demonstrated as compared with c7E3 (table 2). The IC50 values for XV459 or c7E3 in inhibiting platelet aggregation did not significantly differ regardless of whether a single agonist or a combination of agonists such as
Maximal platelet aggregation inhibition was achieved at 50 to $60\%$ receptor occupancy. 

Table 1: Comparative antiplatelet efficacy of XV459 vs. c7E3 in inhibiting human platelet aggregation induced by various agonists

<table>
<thead>
<tr>
<th>Test Agent</th>
<th>IC$_{50}$ ($\mu$M) ± S.E.M.</th>
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<tbody>
<tr>
<td>ADP (100 $\mu$M)</td>
<td>TEAC*</td>
</tr>
<tr>
<td>XV459</td>
<td>0.030 ± 0.0065</td>
</tr>
<tr>
<td>c7E3</td>
<td>0.054 ± 0.0019</td>
</tr>
</tbody>
</table>

* TEAC: thrombin (0.001 IU/ml), epinephrine (100 $\mu$M), ADP (100 $\mu$M) and collagen (20 $\mu$g/ml). ** Thrombin at 0.5 IU/ml. Data represent mean ± S.E.M., n = 3 for each individual agonist.

Platelet Binding Kinetics

Binding affinity to activated vs. unactivated platelets. XV459 binds with high affinity to unactivated and activated human platelets with $K_d = 0.0025 ± 0.0010$ and 0.00080 ± 0.0002 $\mu$M, respectively (fig. 4; table 4). Similarly, the chimeric 7E3 demonstrated a comparable affinity for unactivated and activated human platelets with $K_d = 0.0001 ± 0.0005$ and 0.0092 ± 0.0006 $\mu$M, respectively (table 5). $^3$H-XV459 exhibited comparable high-affinity binding to either activated or unactivated platelets obtained from human, baboon or canine but not swine (table 4). These in vitro binding kinetics of XV459 in various species reflect its in vivo antiplatelet efficacy in those species (data not shown).

Dissociation rates. XV459 has comparable binding affinity to activated and unactivated platelets. It dissociates from unactivated human, baboon or canine platelets at different rates (in vivo) than from activated platelets (in vitro). The rank order of the relative dissociation was baboon < human < canine (fig. 5). These in vitro dissociation rates of XV459 in various species reflect its in vivo duration of antiplatelet efficacy in those species (data not shown).
Degree and extent of association with unactivated platelets. A significant association of XV459 after gel filtration with platelets obtained from human, baboon or canine was evident from the inhibition of $^{125}$I fibrinogen binding to activated platelets (fig. 6). The greater the shift to the right, the greater the degree of platelet association. A variable degree of association, depending on the species, was shown. The rank order of relative association was baboon > human > canine (fig. 6).

Elution profile of radiolabeled GPIIb/IIIa antagonists. Association of radiolabeled GPIIb/IIIa antagonists with unactivated platelets demonstrated a significant association of $^3$H-XV459 or $^{125}$I-c7E3 with human platelets as compared with that of $^3$H-DMP728 (fig. 7).

Discussion

New targets for antiplatelet therapy have been identified on the basis of a better understanding of the processes that lead to platelet-rich thrombus formation. Once endothelial damage occurs, platelet thrombus formation advances in three steps: 1) platelet adhesion, 2) platelet activation by the various agonists or activators from damaged endothelium and from within activated platelets and 3) the resulting aggregation. The final step in thrombus formation is mediated exclusively by the GPIIb/IIIa receptor. Arterial thrombosis has 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 (Fitzgerald et al., 1986; Fuster et al., 1985; Hamm et al., 1987). The contribution of platelets to these disease processes stems from their ability to form aggregates, or platelet thrombi, as a consequence of arterial injury (Fitzgerald et al., 1986; Fuster et al., 1985; Hamm et al., 1987).

It has been recognized that the platelet GPIIb/IIIa, via its binding to circulating fibrinogen, is the final common pathway for all agonists-induced platelets aggregate formation (Philips et al., 1991; Pytela et al., 1986). The binding of fibrinogen is mediated in part by the RGD recognition sequence, which is common to other adhesive proteins that bind to GPIIb/IIIa receptors or other integrins (D’Souza et al., 1990; Philips et al., 1991; Pytela et al., 1986). Various large-scale phase III clinical trials have illustrated the usefulness of ReoPro in percutaneous coronary interventions (The EPIC Investigators, 1994; Topol, 1995). The first such study was the pivotal EPIC trial that enrolled 2099 high-risk patients scheduled to undergo percutaneous intervention. Although the EPIC trial found a significant 35% (13.1% vs. 7.7%, P = .008) reduction in the rate of composite endpoint events in patients treated with Abciximab bolus (0.25 mg/kg i.v.) and infusion (10 µg/min, infusion for 12 hr) at 30 days, it also raised the concern of excess and significant bleeding complications (The EPIC Investigators, 1994). Data analysis from this trial confirms that ReoPro treatment significantly reduced the incidence of death and myocardial infarction (MI) at the 30-day primary endpoint; the greatest effect was observed on the occurrence of large, non-Q-wave MI (Topol, 1997).

Several other selective GPIIb/IIIa antagonists, including Integrin, Tirofiban (MK383) and Lamifiban, are in advanced stages of clinical development, aimed primarily for i.v. use in the treatment and prevention of acute ischemic heart diseases (Peerlinck et al., 1993; Techeng et al., 1995; Topol et al., 1994). IMPACT II, PURSUIT trials with Integrin and RESTORE trial with Tirofiban, as well as many other trials, demonstrated significant clinical benefits in acute ischemic syndromes on the basis of meta-analysis (Topol, 1997). Recent clinical studies with orally active GPIIb/IIIa antagonists including Xemilofiban (SC54684) and Fradafiban (BIBU104) demonstrated oral antiplatelet activity in the human upon their administration two to three times per day (Kottke-Marchant et al., 1995; Narjes et al., 1995).

These factors prompted us to develop a potent and selective GPIIb/IIIa antagonist for treatment of the different thromboembolic disorders. In PRP aggregation, aspirin, ticlopidine and hirudin were shown to be effective mainly against a single agonist (arachidonic acid, ADP and thrombin, respectively) in inhibiting platelet aggregation, with IC$_{50}$ values of 10, 50 and 0.1 µM, respectively (Mousa and Reilly, 1995). Unlike those currently available antiplatelet drugs, XV459 demonstrated high and similar potency in inhibiting platelet aggregation regardless of the agonist or the anticoagulant
used. A lower IC$_{50}$ for XV459 in inhibiting $^{125}$I-fibrinogen binding to purified platelets (GPP) as compared with that required for the inhibition of platelet aggregation in PRP could be due to the effects of plasma RGD containing matrix proteins such as fibrinogen in PRP vs. GPP. XV459 is shown to be a competitive inhibitor with high affinity in inhibiting fibrinogen binding to platelet GPIIb/IIIa receptors (Mousa et al., 1996). Furthermore, XV459 is shown to have an IC$_{50}$ of 0.01 M in inhibiting platelet aggregation in GPP (data not shown) vs. an IC$_{50}$ of 0.024 in inhibiting platelet aggregation in PRP under the same conditions. Comparable IC$_{50}$ values for XV459 or c7E3 in inhibiting platelet aggregation were demonstrated regardless of the agonist or the anticoagulant used. This is in contrast to the significant shift in the IC$_{50}$ of Integrelin in inhibiting platelet aggregation to a greater extent in citrate (relatively lower IC$_{50}$) than in heparin (relatively higher IC$_{50}$) collected blood, which is due to the partial calcium-chelating effect of citrate resulting in artificial enhancement of ex vivo or in vitro Integrelin antiplatelet efficacy (Tcheng, 1997). The implication is that Integrelin might be underdosed in IMPACTII and that greater efficacy might be possible with an upward dose adjustment. This is not the case with other GPIIb/IIIa antagonists with high affinity for both unactivated and activated platelet, such as XV459 and c7E3, as demonstrated in the present study. This is in agreement with earlier studies that demonstrated equipotency of the monoclonal antibody 7E3 in binding to both activated and unactivated human platelets (Coller, 1985). More recent platelet binding studies with the Fab2 fragment c7E3 demonstrated comparable $K_d$ (0.005–0.001 M) of binding to either activated or unactivated human platelets (Personal communication, R. Gordon, Centocor, 1997). We observed comparable high-affinity binding ($K_d = 0.0008–0.0025$ M) for radiolabeled XV459 to either activated or unactivated platelets obtained from human, baboon or canine, but not swine (table 4). These in vitro binding kinetics of XV459 in the various species reflect the in vivo antiplatelet efficacy in those species (Mousa et al., 1997). The binding kinetics were shown to be somewhat comparable in platelets obtained from human, canine and baboon with regard to the $K_d$. A closely similar $K_{on}$ for XV459 was shown in platelets obtained from human and baboon, but not from canine. In contrast, a much weaker affinity for XV459 was

### Table 4

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean $K_d$ (mM) ± S.E.M.</th>
<th>Unactivated</th>
<th>Activated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>0.0025 ± 0.0010</td>
<td>0.0008 ± 0.0002</td>
<td></td>
</tr>
<tr>
<td>Canine</td>
<td>0.0034 ± 0.0014</td>
<td>0.0026 ± 0.001</td>
<td></td>
</tr>
<tr>
<td>Baboon</td>
<td>0.00088 ± 0.00044</td>
<td>0.00049</td>
<td></td>
</tr>
<tr>
<td>Swine</td>
<td>0.1210 ± 0.0275</td>
<td>0.1223 ± 0.0257</td>
<td></td>
</tr>
</tbody>
</table>

Data represent mean ± S.E.M., $n = 3$ to 6, except for baboon, which was based on pooled platelets from three different animals.

### Table 5

<table>
<thead>
<tr>
<th>Binding Parameters</th>
<th>XV459</th>
<th>c7E3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_d$ (µM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unactivated</td>
<td>0.0025 ± 0.000098</td>
<td>0.0001 ± 0.0005</td>
</tr>
<tr>
<td>Activated</td>
<td>0.00008 ± 0.0002</td>
<td>0.0092 ± 0.0006</td>
</tr>
<tr>
<td>Dissociation rates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(½, minutes)</td>
<td>7.0</td>
<td>40.0</td>
</tr>
</tbody>
</table>

Data represent mean ± S.E.M., $n = 3$.
shown for platelets obtained from swine. These binding kinetic profiles ($K_d$ and $K_{off}$) of XV459 allowed for a long-lasting antiplatelet efficacy for up to 24 hr after once-a-day p.o. dosing (Mousa et al., 1997). The in vitro rates of dissociation of XV459 in the various species as shown in table 4 reflect the duration of antiplatelet efficacy of XV459 in those species (i.e., the slower the rate of dissociation, the longer the duration of antiplatelet efficacy). Additionally, 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 (Mousa et al., 1996). This is unlike the linear RGDS peptide, which recognizes multiple integrin receptors.

The high affinity and specificity of DMP754 to the platelet GPIIb/IIIa might be very important for achieving an optimal efficacy/safety ratio. Additionally, XV459 demonstrated high affinity for both activated and unactivated platelets, along with relatively slow dissociation rates, which suggest a possible prolonged duration of in vivo antiplatelet effects that might be longer than DMP728 and closer to ReoPro. This is in contrast to current i.v. platelet GPIIb/IIIa antagonists such as Integrilin, Tirofiban or Lamifiban and DMP728, which have a short duration of antiplatelet effects associated with their relative fast dissociation rates from human platelets (Mousa et al., 1994; Peerlinck et al., 1993; Tcheng et al., 1995; Topol and Plow, 1993; Topol, 1995).

In conclusion, XV459, the active free acid form of DMP754, represents a high-affinity platelet GPIIb/IIIa receptor antagonist with comparable affinity for activated and unactivated human platelets and with relatively slow dissociation rates that suggest extended duration of in vivo antiplatelet efficacy.

References
Bennett JS, Vilaire GO (1979) Exposure of platelet fibrinogen receptors by ADP, epinephrine and sodium arachidonate at 100 μM each. This representative tracing of the qualitative shift in fibrinogen binding after size exclusion chromatography demonstrated a tight association of XV459 with unactivated platelets.

Fig. 6. Effects of pretreatment of unactivated platelets obtained from various species including canine (panel a), baboon (panel b) and human (panel c) on the degree and extent of association as evident from the inhibitory effect on $^{125}$I-fibrinogen binding to activated platelets. Platelets were activated with agonist mixture containing ADP, epinephrine and sodium arachidonate at 100 μM each. This representative tracing of the qualitative shift in fibrinogen binding after size exclusion chromatography demonstrated a tight association of XV459 with unactivated platelets.

Fig. 7. Elution profile of $^3$H-XV459 (panel a), $^{125}$I-c7E3 (panel b) and $^3$H-DMP728 (panel c) with unactivated human platelets upon size exclusion chromatography separation. Data demonstrated a tight and high association of $^3$H-XV459 (panel a) and of $^{125}$I-c7E3 (panel b) as compared with that of $^3$H-DMP728 (panel c).


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