Nonpeptide Glycoprotein IIb/IIIa Inhibitors. 15. Antithrombotic Efficacy of L-738,167, a Long-Acting GPIIb/IIIa Antagonist, Correlates with Inhibition of Adenosine Diphosphate-Induced Platelet Aggregation but not with Bleeding Time Prolongation


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ABSTRACT

The nonpeptide platelet glycoprotein IIb/IIIa antagonist, L-738,167, was characterized in dog and nonhuman primate. In an anesthetized canine model of coronary artery electrolytic lesion, L-738,167 elicited dose-dependent (3, 4, 4.5 and 5 μg/kg i.v.) decreases in incidence of occlusion, reductions in thrombus mass and elevations in bleeding time. Antithrombotic efficacy correlated with inhibition of adenosine diphosphate-induced platelet aggregation but was dissociated from marked bleeding time elevation. Similarly, suppression of platelet-dependent cyclic flow reductions with L-738,167 in the canine coronary artery (5 μg/kg i.v.) and African green monkey carotid artery (10 μg/kg i.v.) correlated with inhibition of adenosine diphosphate-induced platelet aggregation but not with inhibition of thrombin-induced platelet aggregation or significant prolongation of bleeding time. In conscious dogs and sedated chimpanzees, single dose intravenous bolus (5–20 μg/kg) and oral (25–200 μg/kg) administration of L-738,167 exhibited long duration (>8 hr) inhibition of ex vivo platelet aggregation. Once daily oral administration to conscious dogs (10–30 μg/kg/day for 15 days) and rhesus monkeys (200–250 μg/kg/day for 11 days) maintained significant but submaximal (50–90% inhibition) trough levels of inhibition of adenosine diphosphate-induced ex vivo platelet aggregation. Platelet sensitivity to adenosine diphosphate after multiple days of oral dosing in dogs was similar to pretreatment sensitivity. L-738,167 showed characteristics suitable for chronic oral therapy with a glycoprotein IIb/IIIa inhibitor.

Inhibition of platelet GPIIb/IIIa-fibrinogen binding, the final common pathway of platelet aggregation, has emerged as a predominant target in the development of new, more effective antithrombotic agents (Coller et al., 1995; Frishman et al., 1995; Ojima et al., 1995; Topol 1995). Clinical studies have been conducted with i.v. platelet GPIIb/IIIa inhibitors, including the anti-GPIIb/IIIa c7E3 Fab antibody (abciximab, ReoPro) (EPIC Investigators, 1994; Topol et al., 1994; Kleiman et al., 1993; Simoons et al., 1994), integrin, a cyclic peptide inhibitor (Schulman et al., 1993; Harrington et al., 1994; Ohman et al., 1994; Thcheng et al., 1995), and the small molecule nonpeptide inhibitors, tirofiban (Aggrastat) (Kereiakes et al., 1994; Thérioux et al., 1994) and lamifiban (Thérioux et al., 1996) in PTCA, thrombolysis and unstable angina. Results of the initial study with abciximab demonstrated that the platelet GPIIb/IIIa receptor is an effective antithrombotic target (EPIC Investigators, 1994; Topol et al., 1994). Orally active platelet GPIIb/IIIa antagonists would significantly expand the therapeutic use of this class of agents to include the chronic treatment of cardiovascular, cerebrovascular and peripheral vascular thrombotic disorders. Implicit in the identification of a therapeutically useful orally active antplatelet agent are requirements for high oral potency and extended duration of action to minimize the level and frequency of dosing. In addition, a compound with these characteristics would allow expanded understanding of the relationship among the antiaggregatory effect, antithrombotic activity and bleeding time prolongation.

L-738,167 (fig. 1) is an intrinsically active, highly potent nonpeptide antagonist of platelet GPIIb/IIIa (Askew et al., in press; Bednar et al., in press). Complete prevention of coro-

ABBREVIATIONS: ADP, adenosine diphosphate; AGM, African green monkey; CFR, cyclic flow reduction; GP, glycoprotein; LCX, left circumflex coronary artery; PPP, platelet-poor plasma; PRP, platelet-rich plasma; PTCA, percutaneous transluminal coronary angioplasty; TRAP, thrombin receptor activating peptide.
nary artery thrombosis and ensuing myocardial infarction were demonstrated with single dose or once daily multiple dose (with the thrombotic stimulus initiated 12 hr after dosing) oral administration of 100 to 300 μg/kg L-738,167 in a conscious, instrumented canine model of coronary artery electrolytic injury. Lower oral doses of L-738,167, 10 to 30 μg/kg, reduced the incidence of occlusive coronary thrombosis, delayed the time to occlusion and reduced thrombus mass and infarct size in this model in the conscious dog (Cook et al., in press). The aim of our study was to characterize the i.v. and oral potency and duration of antiaggregatory activity of L-738,167, as well as the ability to achieve and maintain submaximal but therapeutic levels of inhibition of platelet aggregation with once daily oral doses of L-738,167 for extended periods. The relationship between antiaggregatory activity and effects on template bleeding time was also evaluated. Finally, the antithrombotic efficacy of L-738,167 was explored to specifically address the relationships among ex vivo antiaggregatory activity in response to multiple agonists, effects on template bleeding time and prevention of in vivo intravascular thrombosis.

Methods

All procedures related to the use of animals in these studies were reviewed and approved by the Institutional Animal Care and Use Committee at Merck Research Laboratories at West Point, PA and conform with the Guide for the Care and Use of Laboratory Animals (U.S. National Institutes of Health Publication no. 85-23, revised 1985).

Single Dose i.v. Administration of L-738,167 to Conscious Dogs and Sedated Chimpanzees

The dose-dependence of antiplatelet activity of L-738,167 was assessed after single dose i.v. administration to conscious dogs and sedated chimpanzees. Three groups of conscious, purpose-bred mongrel dogs of either sex (7.6–15.2 kg) were administered L-738,167 as an intravenous bolus (vehicle = saline) of 5 (n = 6), 10 (n = 6) or 20 (n = 4) μg/kg. During all studies the dogs rested comfortably in nylon slings and at specified time points, blood samples were obtained from either saphenous or cephalic veins (0.38% sodium citrate, final concentration) for the measurement of ex vivo platelet aggregation responses to ADP and collagen and for whole blood platelet counts, as described below. Blood samples were obtained before compound administration (baseline), and at multiple timepoints up to 8 hr after dosing. Chimpanzees (n = 6) were sedated with 10 mg/kg ketamine i.m. for blood withdrawal and intravenous administration of L-738,167. Blood samples were taken before dosing and at multiple timepoints from 1 or 5 min through 32 or 96 hr after dosing for the measurement of ex vivo platelet aggregation responses to ADP and collagen and for whole blood platelet counts, as described below.

Relationship between Acute Effects of Intravenous L-738,167 on Ex Vivo Platelet Aggregation Responses and Bleeding Times in Anesthetized Dogs

The relationship between the inhibition of platelet aggregation and the prolongation of bleeding time was investigated during the intravenous administration of increasing doses of L-738,167 to the anesthetized dog. For the purposes of this study two groups of pentobarbital anesthetized dogs were evaluated for ex vivo inhibition of platelet aggregation and template bleeding time: L-738,167 (4–7 increasing i.v. bolus doses at 30 min intervals, n = 6) and vehicle (5 ml saline, 4 i.v. bolus administrations at 30 min intervals, n = 4). Purpose-bred mongrel dogs of either sex (9.6–17.0 kg) were anesthetized with 35 mg/kg i.v. sodium pentobarbital, intubated, ventilated with room air and instrumented with arterial catheters for blood withdrawal and venous catheters for the infusion of experimental agents and supplemental anesthesia. Before the administration of the first bolus of L-738,167 or vehicle, a blood sample (5 ml) was taken to determine the baseline platelet aggregation response to ADP and collagen as described below, and buccal mucosal bleeding time was measured using a Simplate bleeding time device (Organon Teknika Corporation, Durham, NC) as described below. Bleeding times in this study were measured for a maximum of 15 min. One min after each bolus, a blood sample was obtained and a bleeding time measurement was initiated. A second blood sample was taken at the termination of the bleeding time measurement. For each individual dose, the percent inhibition of aggregation was averaged from the “pre” and “post” bleeding time samples and this value was used to represent the effect on platelet aggregation for that particular dose and measured bleeding time. This was repeated for four to seven i.v. bolus doses of L-738,167 (0.5 μg/kg followed by 0.5 or 1.0 μg/kg, three to six times) or for four i.v. bolus infusions of vehicle. In the L-738,167 treatment group the final dose was not predetermined, but was the dose at which template bleeding time exceeded the maximum limit which was set at 15 min.

Prevention of Acute Thrombus Formation with Intravenous L-738,167 in an Anesthetized Canine Model of LCX Electrolytic Injury and Thrombosis

Male or female purpose-bred mongrel dogs (10.2–13.5 kg) were anesthetized with sodium pentobarbital (35 mg/kg, i.v.) and ventilated with room air using a positive pressure ventilator (Harvard Apparatus, S. Natick, MA). The right femoral artery and the right and left femoral veins were cannulated for the measurement of mean arterial pressure (Statham P23ID, Gould Inc, Cleveland, OH), supplemental anesthesia infusion and drug administration, respectively. The left femoral vein was subsequently used for a continuous infusion of 5% dextrose in saline throughout the course of the experiments. The heart was exposed via a left thoracotomy at the 5th intercostal space. The LCX was isolated proximal to the first obtuse marginal branch, and dissected for a distance of approximately 2 cm. The vessel was instrumented, proximal to distal, as follows: electromagnetic flow probe (model 501, Carolina Medical Electronics Inc, King, NC), stimulation electrode, adjustable mechanical occluder (Goldblatt clamp) and silk snare. The stimulation electrode was constructed from a 26-gauge stainless steel hypodermic needle tip attached to a 30-gauge Teflon-insulated silver-coated copper wire. The mechanical occluder was constructed of stainless steel with a stainless steel screw (2 mm diameter), which could be manipulated to control vessel circumference. The occluder was sufficiently tightened around the artery to just eliminate the reactive hyperemic response without affecting resting LCX blood flow. Continuous records of systemic blood pressure and mean and phasic LCX blood flow were displayed on a model 7E polygraph (Grass Instrument Co., Quincy, MA). Zero flow and hyperemic flows were determined by occluding the circumflex coronary artery distal to the flow probe for 20 sec with the snare ligature. Thirty min after surgical preparation, animals were randomized.
to i.v. bolus administration of normal saline (vehicle, n = 8) or 3.0, 4.0, 4.5 or 5.0 μg/kg L-738,167 (n = 6/group) in 5 ml saline followed by 5 ml saline flush. In this initial standard pretreatment protocol, the process of thrombotic occlusion of the LCX was initiated at 5 min after single dose i.v. treatment by the application of 100 μA continuous anodal direct current to the LCX stimulation electrode for 60 min. Direct electrical stimulation was delivered using a Grass constant current unit (model CCU1A), a Grass stimulus isolation unit (model SIU5) and a Grass stimulator (model S48) connected to the intraluminal LCX stimulation electrode. Intracoronary thrombi were retrieved and residual wet thrombus mass obtained after a 30 min period of zero flow or after 5 hr. Blood samples for the determination of whole blood platelet counts and ex vivo platelet aggregation responses to ADP and collagen, as described below, were obtained at the following timepoints in the protocol: immediately before treatment (baseline) and at 1, 2, 3, 4 and 5 hr after vehicle or L-738,167 treatment. Heart rate, mean arterial pressure and buccal mucosal template bleeding times, as described below, were also determined at these same timepoints. Bleeding times in this study were measured for a maximum of 20 min.

In an extension of the preceding study, the experimental design was modified so that the LCX current was initiated at 3 or 5 hr after single dose i.v. treatment (as opposed to 5 min after treatment in the initial study) to further characterize the relationship among in vivo efficacy, prolongation of bleeding time and inhibition of ex vivo platelet aggregation. Purpose-bred mongrel dogs (10.0–15.4 kg) were randomized into four groups: vehicle control with current on at 3 hr (n = 3); 5 μg/kg L-738,167 with current on at 3 hr (n = 6); vehicle control with current on at 5 hr (n = 3) and 5 μg/kg L-738,167 with current on at 5 hr (n = 6).

**Prevention of LCX CFRs with i.v. L-738,167 in an Anesthetized Canine Model: Relationship of Antithrombotic Efficacy to Inhibition of Platelet Aggregation and Prolongation of Template Bleeding Time**

Purpose-bred mongrel dogs of either sex (9.8–13.0 kg) were surgically prepared as described above for the anesthetized dog electrolytic injury model. For these experiments the vessel was instrumented, proximal to distal, as follows: electromagnetic flow probe (model 501, Carolina Medical Electronics Inc.), a silver clip constrictor and a snare ligature. Physiological parameters were recorded on a model 7D polygraph (Grass Medical Instruments). After instrumentation of the LCX with the silver clip the phasic pattern of blood flow was significantly reduced; however, the mean flow was not changed. CFRs were initiated by application of the silver clip constrictor and inducing endothelial damage with repetitive external pinching of the vessel. The accumulation of platelet aggregates in the vessel lumen was observed as a gradual reduction in blood flow. When flow reached its lowest level, the platelet plug was mechanically dislodged, and blood flow was restored. An initial 15 min of consistent, reproducible CFRs was required for inclusion of each preparation in the study. During this period the range of constricted blood flows that would elicit CFRs, was established. When reapplying the silver clip for the start of each successive CFR, the clip was adjusted to ensure that flow was within the acceptable range of starting flows that had been predetermined for each individual monkey to elicit CFRs. After the 15 min initiation period of baseline CFRs, vehicle (saline, 5 ml bolus) was administered and CFRs were continued for an additional 15 min. After 30 min of acceptable CFRs, 10 μg/kg i.v. L-738,167 was administered in saline (5 ml followed by 5 ml flush).

One group of monkeys (n = 5) participated in this study with each animal serving as its own control. After the administration of L-738,167, 10 μg/kg i.v., CFRs were attempted by pinching the vessel to ensure exposure of thrombogenic subendothelium and repositioning the silver clip constrictor. The attempt to restart CFRs was repeated at 15 min intervals for 180 min after dosing. Blood samples were obtained for measurement of ex vivo platelet aggregation responses to ADP, collagen and thrombin in platelet-rich plasma and thrombin-induced platelet aggregation in whole blood and for whole blood platelet counts, as described below. Template bleeding time (maximum = 20 min) was also determined at the same timepoints as the blood samples: 0.5, 1.5, 2.5, 3.5 and 4.5 hr after dosing (30 min before each attempt to restart CFRs).

**Prevention of Carotid Artery CFRs with i.v. L-738,167 in an Anesthetized African Green Monkey Model: Relationship of Antithrombotic Efficacy to Inhibition of Platelet Aggregation and Prolongation of Template Bleeding Time**

African green monkeys of either sex (3.7–7.1 kg) were sedated with 10 mg/kg i.m. ketamine HCl, anesthetized with 12.5 mg/kg i.v. sodium pentobarbital and ventilated with room air using a positive pressure ventilator (Harvard Apparatus). The right femoral artery and the right and left femoral veins were cannulated for blood collection and the continuous monitoring of hemodynamic parameters (Statham P23ID, Gould Inc, Cleveland, OH), supplemental anesthesia infusion and compound administration, respectively. The left femoral vein was subsequently used for a continuous infusion of 5% dextrose in saline throughout the course of the experiments. A 4 cm segment of the left carotid artery was isolated and instrumented from proximal to distal with an electromagnetic flow probe (model 501, Carolina Medical Electronics Inc.), a silver clip constrictor and a snare ligature. Physiological parameters were recorded on a model 7D polygraph (Grass Medical Instruments). After instrumentation of the carotid artery with the silver clip the phasic pattern of blood flow was significantly reduced; however, the mean flow was not changed. CFRs were initiated by application of the silver clip constrictor and inducing endothelial damage with repetitive external pinching of the vessel. The accumulation of platelet aggregates in the vessel lumen was observed as a gradual reduction in blood flow. When flow reached its lowest level, the platelet plug was mechanically dislodged, and blood flow was restored. An initial 15 min of consistent, reproducible CFRs was required for inclusion of each preparation in the study. During this period the range of constricted blood flows that would elicit CFRs, was established. When reapplying the silver clip for the start of each successive CFR, the clip was adjusted to ensure that flow was within the acceptable range of starting flows that had been predetermined for each individual monkey to elicit CFRs. After the 15 min initiation period of baseline CFRs, vehicle (saline, 5 ml bolus) was administered and CFRs were continued for an additional 15 min. After 30 min of acceptable CFRs, 10 μg/kg i.v. L-738,167 was administered in saline (5 ml followed by 5 ml flush).

One group of monkeys (n = 5) participated in this study with each animal serving as its own control. After the administration of L-738,167, 10 μg/kg i.v., CFRs were attempted by pinching the vessel to ensure exposure of thrombogenic subendothelium and repositioning the silver clip constrictor. The attempt to restart CFRs was repeated at 15 min intervals for 180 min after dosing. Blood samples were obtained for measurement of ex vivo platelet aggregation responses to ADP, collagen and thrombin in platelet-rich plasma and thrombin-induced platelet aggregation in whole blood and for whole blood platelet counts, as described below. Forearm template bleeding time also was measured at the following timepoints: baseline, pre-L-738,167 (vehicle) and 0.5, 2 and 3 hr after dosing, as described below.

**Single Dose Oral Administration of L-738,167 by Gastric Lavage or Capsule to Conscious Dogs**

The dose-dependence of oral antiplatelet activity of L-738,167 was assessed after single dose oral administration in aqueous solution by gastric lavage to conscious dogs. Six groups of conscious, purpose-bred mongrel dogs of either sex (7.5–18.3 kg) were administered
L-738,167 orally by gastric lavage in aqueous solution (5 ml volume, vehicle = sterile water, followed by a 10 ml flush): 15 μg/kg (n = 4); 25 μg/kg (n = 5); 50 μg/kg (n = 6); 100 μg/kg (n = 3); 200 μg/kg (n = 2) and vehicle (n = 4). For all six treatment groups described above, blood samples were obtained before compound administration (baseline), and at multiple timepoints up to 8 hr after compound administration. A separate group was administered L-738,167 orally as solid material (crystalline) in gelatin capsule at 100 μg/kg; blood samples were obtained before compound administration (baseline), and at 0.33, 2.5, 24, 48, 72 and 96 hr after dosing.

Once Daily Oral Administration of L-738,167 for 15 Days to Conscious Dogs by Gastric Lavage in Aqueous Solution: Maintenance of Inhibition of Platelet Function and Effect on Platelet Sensitivity

A 15 day study in dogs was conducted to determine if submaximal but therapeutic levels of inhibition of platelet aggregation could be maintained with once daily oral doses of L-738,167. A group of conscious, purpose-bred mongrel dogs of either sex (n = 6, 7.6–11.0 kg) was administered individualized loading doses of 50 to 90 μg/kg L-738,167 on the first day of treatment (day 0), followed by once daily doses of either 10 or 30 μg/kg L-738,167 for 14 consecutive days (days 1–14). L-738,167 was administered in solution by gastric lavage (5 ml volume with sterile water as vehicle and 10 ml flush with sterile water). Blood samples were obtained before the first oral dose (baseline), at 120 min after the first oral dose and at 30 min before compound administration on succeeding days (i.e., 23.5 hr after dosing on the previous day) for the measurement of ex vivo platelet aggregation responses to ADP and collagen and for whole blood platelet counts, as described below. Blood samples also were obtained on the 5 consecutive days after termination of once daily oral dosing (days 15–19) for the measurement of ex vivo platelet aggregation responses to ADP and collagen and for whole blood platelet counts. In addition, the reactivity of the platelets of five of these dogs to ADP was determined by measuring the response of platelets in platelet counts. In addition, the reactivity of the platelets of five of these dogs to ADP and collagen and for whole blood platelet counts, as described below. Blood samples also were obtained on the 5 consecutive days after termination of once daily oral dosing (days 15–19) for the measurement of ex vivo platelet aggregation responses to ADP and collagen and for whole blood platelet counts. In addition, the reactivity of the platelets of five of these dogs to ADP was determined by measuring the response of platelets in platelet counts.

Once Daily Oral Administration of L-738,167 for 11 Days to Conscious Rhesus Monkeys by Nasogastric Lavage in Aqueous Solution

Similar to the study described above in dogs, an 11 day study in monkeys was conducted to determine if submaximal but therapeutic levels of inhibition of platelet aggregation could be maintained in primates with once daily oral doses of L-738,167. Conscious rhesus monkeys, either sex (n = 4, 3.9–6.05 kg) were administered a loading dose of 300 μg/kg L-738,167 on the first day of treatment (day 0), followed by 3 days (days 1–3) of once daily maintenance doses of 200 μg/kg and 7 days (days 4–10) of once daily maintenance doses of 250 μg/kg L-738,167. Monkeys were placed in primate restraint chairs for oral dosing and blood withdrawal. L-738,167 was administered in solution by nasogastric lavage (10 ml volume with sterile water as vehicle followed by 5 ml flush with sterile water). Blood samples were obtained from the saphenous vein (0.38% sodium citrate, final concentration) before the first oral dose (baseline), at 180 min after the first oral dose and at 30 min before compound administration on succeeding days (i.e., 23.5 hr after dosing on the previous day) for the measurement of ex vivo platelet aggregation responses to ADP and collagen and for whole blood platelet counts, as described below. Blood samples also were obtained on day 13 (i.e., 3 days after termination of once daily oral dosing) for the measurement of ex vivo platelet aggregation responses to ADP and collagen and for whole blood platelet counts.

Measurement of Hematologic Parameters

Ex vivo platelet aggregation responses to ADP, collagen and thrombin in PRP in dogs and nonhuman primates. Blood was withdrawn into sodium citrate (0.38%, final concentration). PRP was prepared by centrifugation of whole blood at 150 × g for 5 min and the platelet concentration was adjusted to 2 × 10⁸ platelets/ml with time-matched PPP. PRP (300 μl, 2 × 10⁸ platelets/ml) was incubated at 37°C for 3 min before the addition of agonist. Platelet aggregation was measured by the change in light transmittance (PPP represents 100%) under stirring conditions (1100 rpm) at 37°C in a Biodata Platelet Aggregation Profiler, model PAP-4 (Horsham, PA). In dogs, aggregation was initiated by the addition of 10 μM ADP + 1 μM epinephrine, 10 μg/ml collagen + 1 μM epinephrine or 20 nM thrombin + 1 μM epinephrine. Epinephrine was used to enhance the aggregation response of canine platelets to other agonists. In monkeys and chimps, aggregation was initiated with 20 μM ADP, 10 μg/ml collagen or 100 nM thrombin. The extent of aggregation is reported as the peak percent of aggregation achieved based on a maximum of 100% and the rate of aggregation was determined from the maximum slope of the aggregatory response. The effect of L-738,167 treatment on the extent and rate of aggregation is expressed as the percent inhibition using the baseline, pretreatment aggregation response (day 0 in multiple day maintenance studies) as 100%.

Thrombin-induced whole blood platelet aggregation in dogs and nonhuman primates. Whole blood, 0.4 ml, was incubated with H₂N-Gly-Pro-Arg-Pro-OH peptide for 3 min at 37°C under stirring conditions (1100 rpm). GRP was added to prevent thrombin-mediated fibrin polymerization. Thrombin (dog, 20 nM; African green monkey, 100 nM) was added to the stirred sample, and after an additional 4 min, a platelet count was determined with an automated hematology analyzer (Seronox-Baker Diagnostics, Allen-town, PA). A decrease in the number of single platelets was used to measure the extent of platelet aggregation.

Whole blood platelet counts in dogs and nonhuman primates. Whole blood platelet counts were determined using an automated hematology analyzer (Seronox-Baker Diagnostics).

Buccal mucosal template bleeding times in dogs. Buccal mucosal template bleeding times were measured with a Simplate bleeding time device (Organon Teknika Corporation, Durham, NC). Uniform incisions were made on the mucous membrane of the inner upper lip of the dog, and the duration of bleeding was timed to a maximum of 15 to 20 min as indicated in each study.

Forearm template bleeding times in nonhuman primates. Template bleeding times were measured with a Simplate bleeding time device (Organon Teknika Corporation). A blood pressure cuff was placed on the upper arm and inflated to 50 mm Hg; uniform incisions were made on the muscular part of the forearm and the duration of bleeding was timed to a maximum of 20 min.

Statistical Analysis

Data are expressed as the mean ± S.E.M. Among group comparisons were primarily performed using a two-way analysis of variance, followed by a Dunnett’s test of all pairwise comparisons; an unpaired Student’s t test was used for comparisons between two groups. Within group comparisons were performed using a one-way analysis of variance with repeated measures, followed by a Dunnett’s test for multiple comparisons to one control (baseline) group. The Fisher’s exact test was used for among group comparisons in the “incidence of occlusion.” For all statistical analyses, differences at the level of P < .05 were considered significant.

Results

Single dose i.v. administration of L-738,167 to conscious dogs and sedated chimpanzees. The effects of the single dose i.v. bolus administration of 5, 10 and 20 μg/kg
L-738,167 on ex vivo platelet aggregation responses to ADP (extent) in conscious dogs is depicted in figure 2A. In the lowest dose group, 5 μg/kg, platelet aggregation responses to ADP varied among the individual dogs and were either completely inhibited (n = 4) or only very minimally reduced (n = 2). The average maximal inhibition of the platelet aggregation response to ADP was 67 ± 20%, observed at 5 min after dosing. The duration of the ex vivo antiplatelet effect in sensitive animals was prolonged, with four of the six dogs in this group displaying nearly complete inhibition of platelet function, and average inhibition of platelet function maintained between 55 to 65% for the 8 hr duration of the protocol. The i.v. administration of 10 μg/kg L-738,167 elicited average maximal inhibition of platelet aggregation response to ADP of 79 ± 13% at 1 min after treatment (four of six dogs completely inhibited). The duration of inhibition of ex vivo platelet response to ADP was prolonged in all animals in this group, and was maintained at an average of 66 to 76% for the 8 hr duration of the study. Administration of the highest dose intravenous bolus L-738,167, 20 μg/kg, resulted in complete inhibition of the platelet aggregation response to ADP for the 8 hr duration of the protocol in all animals in this group. Quantitatively similar profiles of inhibition of ex vivo platelet aggregation were observed when the rate of ADP-induced aggregation and the rate and extent of collagen-induced aggregation were monitored. Whole blood platelet counts were not altered by the intravenous bolus administration of 5 to 20 μg/kg L-738,167 during the course of these 8 hr experiments.

The i.v. bolus administration of 10 μg/kg L-738,167 to chimpanzees also resulted in extended inhibition of ex vivo ADP- and collagen-induced platelet aggregation. Complete inhibition was observed immediately after dosing (1 min = 100 ± 0%) that remained for 12 hr (ADP) or 8 to 10 hr (collagen). ADP-induced platelet aggregation was approximately 50% inhibited at 32 hr (extent = 50 ± 10%; rate = 51 ± 9%, n = 6) and platelet function returned to within 20% of baseline by 48 to 96 hr. The rate of collagen-induced platelet aggregation was inhibited through 24 hr (46 ± 5%); however, the extent of collagen-induced aggregation had returned to within 20% of baseline by 24 hr (12 ± 5% inhibition).

**Relationship between effects of i.v. L-738,167 on ex vivo platelet aggregation responses and bleeding times in anesthetized dogs.** The effect of increasing i.v. bolus doses of L-738,167 on template bleeding times relative to inhibition of the extent of ex vivo platelet aggregation induced by ADP in anesthetized dog is depicted in figure 2B. Inhibitions of platelet aggregation of 82 and 94% corresponded to 2.6- and 4.2-fold elevations in bleeding time, respectively. Although complete inhibition of ADP-induced platelet aggregation was achieved after several bolus infusions, additional L-738,167 was required for the prolongation of bleeding time to the maximum time allowed in this protocol (15 min).

**Prevention of acute thrombus formation with i.v. L-738,167 in an anesthetized canine model of LCX electrolytic injury and thrombosis.** Table 1 summarizes the incidence of occlusion, time to the development of occlusive LCX thrombus and residual thrombus mass in saline control and L-738,167 treatment groups in an anesthetized canine model of LCX electrolytic injury. In this initial standard pretreatment protocol, single dose i.v. L-738,167 was administered 5 min before initiation of coronary artery injury. All saline-treated control animals (8/8) developed occlusive LCX thrombi at 74.6 ± 10.0 min after the application of electrical current to the coronary artery, with a residual thrombus mass of 14.8 ± 1.4 mg. The administration of the lowest L-738,167 dose, 3.0 μg/kg i.v., resulted in a modest delay in the formation of occlusive thrombus; however, the residual thrombus mass was reduced significantly to 8.6 ± 1.5 mg, P < .05 vs. saline (table 1). The administration of 4.0, 4.5 and 5.0 μg/kg i.v. L-738,167 resulted in a dose-dependent de-
crease in the incidence of occlusion, increase in time to occlusion and decrease in residual thrombus mass. Treatment with 5.0 μg/kg L-738,167 resulted in complete efficacy and residual thrombus mass was reduced to 3.8 ± 1.3 mg at 5 hr after initiation of injury (table 1). Inhibition of ex vivo platelet aggregation (ADP extent) was >90% in the 3.0 μg/kg i.v. group and was essentially complete in the 4.0, 4.5 and 5.0 μg/kg i.v. groups through the 5 hr time course of the study (fig. 3A). Buccal mucosal template bleeding times were prolonged dose and time dependently with single dose i.v. L-738,167 (fig. 3B). In all L-738,167 groups, template bleeding times decreased toward baseline values during the 5 hr time course of the study (fig. 3B). There were no significant treatment-related effects on heart rate, mean arterial pressure or whole blood platelet count in this study.

Because of the dissociation of bleeding time and inhibition of platelet aggregation at later times after i.v. bolus administration, an additional study was done with the LCX current initiated at 3 or 5 hr after single dose i.v. treatment. For each protocol, animals were randomized to pretreatment with i.v. vehicle (saline, n = 3) or single dose 5 μg/kg i.v. L-738,167 (n = 6). Table 2 summarizes the incidence and time to the development of occlusive coronary thrombus as well as residual thrombus mass in saline control and L-738,167 treatment groups. All vehicle control animals in both the 3 and 5 hr protocols (three of three each) displayed complete coronary occlusion at 41.0 ± 8.3 and 74.0 ± 1.2 min, respectively. Residual thrombus mass in the 3 and 5 hr control groups were 19.9 ± 2.4 and 15.4 ± 2.2 mg, respectively. Pretreatment with single dose 5 μg/kg i.v. L-738,167 at 3 or 5 hr before initiation of injury reduced the incidence of occlusive thrombus formation to four of six and two of six respectively, and in both protocols significantly delayed time to occlusion and reduced residual thrombus mass compared to matched controls (table 2). Buccal mucosal template bleeding times in the 3 hr protocol were elevated only 3.5 ± 0.6-, 2.2 ± 0.3- and 1.5 ± 0.2-fold over baseline at 0.5, 2 and 3.5 hr after initiation of LCX injury. Further, in the 5 hr protocol, template bleeding times were elevated only 2.4 ± 0.4 at the time of initiation of coronary injury, and were 1.7 ± 0.2- and 1.3 ± 0.1-fold over

**Fig. 3.** A, Effects of L-738,167, single dose i.v. vehicle (□), 3.0 (●), 4.0 (◇), 4.5 (○) and 5.0 (▲) μg/kg, on the ex vivo platelet aggregation response (extent) to ADP (10 μM + 1 μM epinephrine) in PRP expressed as percent inhibition in the anesthetized dog LCX electrolytic injury model. Inhibition of platelet aggregation was statistically different from the control group in all L-738,167 treatment groups at all postdose timepoints. B, Effects of L-738,167, single dose i.v. vehicle (□), 3.0 (●), 4.0 (◇), 4.5 (○) and 5.0 (▲) μg/kg, on buccal mucosal template bleeding times in the anesthetized dog LCX electrolytic injury model. Baseline buccal mucosal template bleeding times were: 3.0 μg/kg, 2.42 ± 0.24 min; 4.0 μg/kg, 2.33 ± 0.17 min; 4.5 μg/kg, 2.33 ± 0.21 min; 5.0 μg/kg, 2.08 ± 0.15 min. Data are mean ± S.E.M. with n = 6. *Statistically different from control group, P < .05.
baseline at 1 and 2.5 hr after initiation of current. As was seen previously, ADP-induced platelet aggregation was fully inhibited. Therefore, in this study using a delay in initiation of LCX injury relative to treatment, antithrombotic efficacy was observed with L-738,167 despite only modest elevations in bleeding time. There were no significant treatment-related effects on heart rate, mean arterial pressure or whole blood platelet count in this study.

Prevention of LCX CFRs with i.v. L-738,167 in an anesthetized canine model: relationship of antithrombotic efficacy to inhibition of platelet aggregation and prolongation of template bleeding time. A second type of model was used to further explore this relationship between bleeding time prolongation and inhibition of platelet aggregation. The effects of i.v. vehicle (saline) and single dose i.v. 3.0, 4.0 and 5.0 μg/kg L-738,167 on CFR frequency in the LCX of anesthetized dogs are depicted in figure 4A. In the vehicle-control group, CFRs occurred consistently (CFR frequency = six to seven cycles/15 min) in response to the application of the silver clip constrictor and induction of endothelial damage once each hour throughout the 5 hr duration of the protocol. The single dose i.v. administration of all three doses of L-738,167 resulted in immediate complete abolishment of CFRs. The duration of suppression of CFRs by i.v. L-738,167 was dose-dependent, with the highest dose (5.0 μg/kg) completely preventing the reinduction of CFRs for 3 hr after treatment; CFR frequency compared to the vehicle control group at 4 to 5 hr after treatment remained significantly reduced. Figure 4B compares the effects of 5.0 μg/kg L-738,167 on buccal mucosal template bleeding times to inhibition of ex vivo platelet aggregation to ADP and thrombin (extent) in platelet-rich plasma as well as to thrombin in whole blood. Template bleeding time was maximally prolonged (7.7 ± 0.7-fold over baseline) at 30 min after single dose 5.0 μg/kg i.v. L-738,167 administration, but progressively declined to 1.7 ± 0.2-fold over baseline at 4.5 hr after treatment. Inhibition of platelet response to ADP in platelet-rich plasma was complete throughout the duration of the protocol. Inhibition of platelet response to thrombin in platelet-rich plasma was 90 ± 3% at 30 min after treatment and declined gradually to 49 ± 6% at 4.5 hr after treatment. Inhibition of platelet aggregation to thrombin in whole blood

### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline Vehicle (3 hr)</th>
<th>5.0 μg/kg L-738,167 (3 hr)</th>
<th>Saline Vehicle (5 hr)</th>
<th>5.0 μg/kg L-738,167 (5 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of occlusion</td>
<td>3/3</td>
<td>4/6</td>
<td>3/3</td>
<td>2/6</td>
</tr>
<tr>
<td>Time to occlusion (min)</td>
<td>41.0 ± 8.3</td>
<td>160.8 ± 27.8*</td>
<td>74.0 ± 1.2</td>
<td>159.0 ± 16.4*</td>
</tr>
<tr>
<td>Thrombus mass (mg)</td>
<td>19.9 ± 2.4</td>
<td>6.5 ± 0.9*</td>
<td>15.4 ± 2.2</td>
<td>7.5 ± 1.9*</td>
</tr>
</tbody>
</table>

Data are mean ± S.E.M. Occlusion time is time from initiation of LCX injury current.  
*P < .05 vs. 3 hr saline vehicle by unpaired Student’s t test.  
*P < .05 vs. 5 hr saline vehicle by unpaired Student’s t test.

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**Fig. 4.** A, Effect of L-738,167, single dose i.v. vehicle (●), 3.0 (●), 4.0 (●) or 5.0 (▲) μg/kg, on the frequency of left circumflex coronary artery cyclic flow reductions (CFR, cycles/15 min) in anesthetized dogs. Data are mean ± S.E.M. with n = 8/group. *Statistically different from baseline CFRs in same group, P < .05. When symbols overlap the asterisk represents statistical significance in all groups. B, Effect of L-738,167, single dose i.v. 5.0 μg/kg, on the ex vivo platelet aggregation response (extent) to ADP (●) (10 μM + 1 μM epinephrine) in platelet-rich plasma, to thrombin (○) (20 nM) in platelet-rich plasma and to thrombin (●) (20 nM) in whole blood and on buccal mucosal template bleeding time (●). Open symbols represent effects on platelet aggregation and closed symbols show bleeding time results. Values from anesthetized dogs in CFR study (A). Baseline buccal mucosal template bleeding time = 2.5 ± 0.2 min. Data are mean ± S.E.M. with n = 8.
was complete at 1 min after treatment, but declined rapidly to <15% at 2.5 hr. There were no significant treatment-related effects on whole blood platelet count.

**Prevention of carotid artery CFRs with i.v. L-738,167 in the anesthetized African green monkey: relationship of antithrombotic efficacy to inhibition of platelet aggregation and prolongation of template bleeding time.** The effects of a single i.v. dose of L-738,167 (10.0 μg/kg) on CFR frequency in the carotid artery of anesthetized African green monkeys is depicted in figure 5A. During the baseline, control period (15 min) and after the administration of vehicle (saline), CFRs occurred consistently in response to vessel damage and constriction (CFR frequency = 9.6 ± 0.5 and 9.8 ± 0.2 cycles/15 min, respectively). The single dose i.v. administration of 10.0 μg/kg L-738,167 resulted in immediate and complete abolishment of CFRs which lasted for the entire duration of the 3 hr protocol in all monkeys. Figure 5B compares the effects on forearm template bleeding times to inhibition of ex vivo platelet aggregation to ADP and thrombin (extent) in platelet-rich plasma as well as to thrombin in whole blood. Template bleeding time was maximally prolonged (14.6 ± 1.3-fold over baseline) at 30 min after L-738,167 administration, but declined to 2.5 ± 0.6-fold over baseline at 3 hr after treatment. Inhibition of platelet response to ADP in platelet-rich plasma was near complete through 2 hr of the protocol, and was 87 ± 5% at 3 hr after treatment. Inhibition of platelet response to thrombin in platelet-rich plasma was maintained between 85 to 90% during the 3 hr protocol. Inhibition of platelet aggregation to thrombin in whole blood was 93 ± 2% at 30 min after treatment and declined gradually to 52 ± 13% at 3 hr after treatment. There were no significant treatment-related effects on whole blood platelet count.

**Single dose oral administration of L-738,167 by gastric lavage or capsule to conscious dogs.** The single dose oral administration of 15 to 200 μg/kg L-738,167 by gastric lavage in aqueous solution on the platelet aggregation response in conscious dogs resulted in significant dose-dependent inhibition of ex vivo platelet function; inhibition of the extent of ADP-induced aggregation was depicted in figure 6A. Maximal inhibitions of ADP-induced platelet aggregation and the time point at which peak activity was first observed were as follows: 15 μg/kg (23%, 480 min); 25 μg/kg (50%, 350 min); 50 μg/kg (73%, 480 min); 100 μg/kg (100%, 150 min) and 200 μg/kg (100%, 70 min). The duration of inhibition of ex vivo platelet function was prolonged and maintained throughout the 8 hr protocol for all oral doses of L-738,167. Quantitatively similar profiles of inhibition of ex vivo platelet aggregation were observed with the rate of ADP- or collagen-induced aggregation. Inhibition of the extent of ex vivo platelet aggregation induced by collagen also was dose dependent and prolonged in duration, but slightly lesser in magnitude than that displayed by ADP (extent and rate) and collagen (rate). To determine the duration of effect after the oral administration of L-738,167, 100 μg/kg was administered orally by capsule to conscious dogs and ex vivo platelet aggregation was monitored for 4 days. As shown in figure 6B, platelet function returned gradually over the 4 day period. Whole blood platelet counts were not altered by the oral administration of 15 to 200 μg/kg of L-738,167.

**Once daily oral administration of L-738,167 for 15 days to conscious dogs by gastric lavage in aqueous solution: maintenance of inhibition of platelet function and effect on platelet sensitivity.** To determine if the extended duration of L-738,167 would lead to desensitization or accumulation of effect, trough levels of inhibition of the extent of ADP-induced ex vivo platelet aggregation were monitored during 15 days of once daily oral dosing with L-738,167 in aqueous solution in conscious dogs (fig. 7). The results of this extended maintenance study demonstrated that despite normal variability among individual animals, submaximal levels of inhibition of platelet function could be
maintained with one of two once daily oral doses (10 or 30 mg/kg) of L-738,167. In addition, platelet function as monitored by platelet aggregation returned to normal with a similar or slightly faster time course as after a single oral dose (fig. 6B). Similar profiles of inhibition of ex vivo platelet aggregation were observed with the rate of ADP- or collagen-induced aggregation. Inhibition of the extent of ex vivo platelet aggregation induced by collagen also was well maintained during the 15 day period of once daily oral dosing, but was slightly lesser in magnitude than ADP (extent and rate) and collagen (rate).

The effect of prolonged exposure to L-738,167 on platelet sensitivity to an endogenous agonist was assessed by determining the reactivity of the platelets to ADP at five time points: before the start of the study, during once daily oral maintenance dosing (day 11) and three times after the termination of dosing (table 3). During oral dosing (day 11) there was no response to up to 100 μM ADP. Whole blood platelet counts at the end of the study were not statistically different from the initial baseline platelet count (382 ± 36 vs. 428 ± 43 thousand/μl, respectively).

Once daily oral administration of L-738,167 for 11 days to conscious rhesus monkeys by nasogastric lavage in aqueous solution. As in dogs (above), the results of this extended maintenance study in rhesus monkeys demonstrated that submaximal levels of inhibition of platelet function (70–90% inhibition of extent of ADP-induced aggregation) could be maintained with once daily oral administration (250 mg/kg) of L-738,167 and whole blood platelet counts were not altered by multiple oral doses (data not shown).

Discussion

Basic desirable characteristics for an orally administered GPIIb/IIIa antagonist for chronic therapy include potent inhibition of fibrinogen binding to platelets and adequate duration of activity for once or twice daily dosing. Intravenous GPIIb/IIIa inhibitors such as abciximab (EPIC Investigators, 1994; Simoons et al., 1994; Topol et al., 1994), tirofiban (Kereiakes et al., 1994; Théroux et al., 1994), lamifiban (Théroux et al., 1996) and integrelin (Schulman et al., 1993; Harrington et al., 1995; Ohman et al., 1994; Tcheng et al., 1995), have demonstrated favorable efficacy in reducing the incidence of
ischemic events in unstable angina and in preventing adverse events after PTCA. Of these agents only the anti-GPIIb/IIa c7E3 Fab antibody (abciximab) provides extended duration antiplatelet protection (Tcheng et al., 1995) even though free antibody clears rapidly from the plasma (Genetilla and Mauaro, 1996). This longer duration antiaggregatory activity appears to be related to tight binding of the antibody to the platelet surface that does not allow the antibody to be released until the platelet is cleared from the body (Christopolous et al., 1993). The cyclic peptide, integrin (Harrington et al., 1994; Tcheng et al., 1995), and the small molecule nonpeptides, lamifiban (Jones et al., 1993; Theroux et al., 1996) and tirofiban (Barrett et al., 1994; Peerlinck et al., 1993), show rapidly reversible antiplatelet activity that correlates with rapid clearance from the plasma. These short-acting, rapidly cleared i.v. compounds offer the advantage of almost immediate return to full platelet function when required for bleeding arrest or sudden, repeat surgical intervention. However, there is a need for either a very potent or a highly orally bioavailable compound with extended duration of action to satisfy the requirements of low dosage and low frequency of administration for oral dosing.

A major concern of the chronic administration of potent antiplatelet agents such as GPIIb/IIa antagonists is the risk of critical bleeding. Early clinical studies with i.v. GPIIb/IIa antagonists in PTCA patients found substantial increases in the rate of major bleeding events and transfusions required (EPIC Investigators, 1994) and significantly increased bleeding at vascular access sites (Tcheng et al., 1995). Therapeutic doses of a potent nonpeptide GPIIb/IIa inhibitor in unstable angina patients increased bleeding time (~6- to 8-fold over baseline) and bleeding complications when inhibition of platelet aggregation was >80% (Theroux et al., 1996). However, numerous preclinical animal studies with a variety of GPIIb/IIa antagonists have demonstrated a dissociation between significant template bleeding time prolongation and either ex vivo antiaggregatory activity or in vivo antithrombotic efficacy (e.g., Coller et al., 1989; Lynch et al., 1995; Cook et al., 1996). Our initial experiments (fig. 2B) conducted in dogs to study this relationship showed that significant inhibition (>80%) of ex vivo platelet aggregation was achieved with increasing i.v. bolus doses of L-738,167 with moderate elevations in template bleeding time (2.7-fold over baseline). Thus, this compound, along with others previously reported (Coller et al., 1989; Lynch et al., 1995; Cook et al., 1996), demonstrated that significant levels of inhibition of platelet aggregation could be maintained without dramatic increases in template bleeding time. In addition, oral antithrombotic efficacy was shown in the conscious instrumented dog model of LCX electrolytic injury with moderate elevations in template bleeding time (Cook et al., in press). Although the predictive value of prolonged bleeding time for risk of hemorrhage or surgical bleeding is not proven (Rodgers et al., 1990; Lind, 1991), elevated bleeding time is an indication of compromised hemostatic function.

A major objective of our study was to better understand the relationships among critical parameters used to evaluate efficacy and safety of antiplatelet agents such as template bleeding time, ex vivo platelet aggregation and in vivo antithrombotic efficacy, in canine and nonhuman primate animal models. Results of an earlier study with the 7E3 F(ab')2 GPIIb/IIa antibody using an arterial thrombosis model in nonhuman primates demonstrated that prevention of CFRs could be achieved with less GPIIb/IIa receptor blockade than was required for abolition of platelet aggregation. It was also suggested that preservation of even fewer functional receptors might be adequate to maintain nearly normal bleeding time (Coller et al., 1989). Our studies were designed to expand upon the findings with the 7E3 antibody by the examination of a small molecule nonpeptide GPIIb/IIa antagonist, by examination of ex vivo platelet aggregation initiated by the potent agonist thrombin along with ADP and collagen and by comparison of the relative effects in multiple species using several thrombosis models. The canine thrombosis model of electrolytic injury in the coronary artery was used to study the effects of small incremental increases in i.v. dose to examine the relationship among distinct levels of inhibition of platelet aggregation, template bleeding time and in vivo antithrombotic efficacy. Results showed dose-dependent effects on arterial occlusion, thrombus mass, template bleeding time and ex vivo platelet aggregation. The significant inhibition of aggregation that was elicited by L-738,167 at the initial postdose sampling time was maintained for the entire 5 hr protocol. However, the effect on template bleeding time peaked at the initial timepoint (1 hr postdose) and gradually returned to control values within 5 hr (fig. 3B). This separation of effects on bleeding time and platelet aggregation prompted us to modify the protocol to examine the effects of L-738,167 under conditions in which aggregation was inhibited but bleeding time was not significantly prolonged. For this purpose, we initiated the electrical injury to the vessel either 3 or 5 hr after i.v. bolus administration of 5 μg/kg L-738,167. Effects on ex vivo platelet aggregation and template bleeding time in this study were similar to those in the standard protocol. That is, platelet aggregation was completely inhibited for the duration of the study and bleeding time peaked at the initial post bolus timepoint and gradually returned to control values. Coronary blood flow profiles reflected the delay in coronary artery occlusion along with a reduction in thrombus mass by 5 μg/kg of L-738,167 (table 2). In this modified protocol, the surgical preparation was performed after the administration of compound and there were no problems encountered in the control of surgical bleeding or the maintenance of physiological hemodynamic conditions. These results demonstrate that this compound provides protection against intravascular thrombus formation after bleeding time has returned to baseline and, based on other results (Prueksaritanont et al., in press), after free drug has cleared from the plasma.

Based on the results of the standard and modified canine

| TABLE 3
| Effect of 15 days of once daily oral dosing, L-738,167, 30 μg/kg, on platelet sensitivity to ADP in conscious dogs |

<table>
<thead>
<tr>
<th>Extent of ADP-Induced Aggregation ED50 (μM)</th>
<th>Rate of ADP-Induced Aggregation ED50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>10.4 ± 2.9</td>
</tr>
<tr>
<td>Day 11 during dosing</td>
<td>&gt;100</td>
</tr>
<tr>
<td>9 days postdosing</td>
<td>12.3 ± 4.0</td>
</tr>
<tr>
<td>15 days postdosing</td>
<td>8.0 ± 1.8</td>
</tr>
<tr>
<td>23 days postdosing</td>
<td>11.7 ± 4.1</td>
</tr>
</tbody>
</table>

Data are mean ± S.E.M., n = 5. Postdosing determinations were made 9, 15 and 23 days after the final day of oral dosing.
coronary artery electrical injury models of thrombosis, the model of platelet-dependent cyclic flow reductions in the canine coronary artery (Aiken et al., 1979; Folts et al., 1990) was modified so that CFRs would be initiated at multiple timepoints designed to coincide with complete inhibition of aggregation and varying levels of prolongation of bleeding time. This allowed us to more precisely examine the prolongation of bleeding time that coincided with in vivo antithrombotic efficacy. Results showed that CFRs were inhibited by all doses of L-738,167 (3, 4 and 5 μg/kg i.v.) immediately after dosing and that the time course of the return of CFRs initiated at 1-hr intervals was dose related (fig. 4A). There was a separation of effects on platelet aggregation (ADP) and bleeding time, as described above; i.e., ADP-induced platelet aggregation remained inhibited and bleeding time returned toward baseline values over time. However, the effects on aggregation induced by the more potent platelet agonist, thrombin, in both PRP and whole blood decreased over time (fig. 5B). In addition to these findings in dog, a study was conducted in African green monkeys to determine if the relationship among effects on bleeding time, platelet aggregation and in vivo efficacy seen in dog was consistent in non-human primates. Results showed that L-738,167 (10 μg/kg i.v.) completely inhibited carotid artery CFRs, bleeding time was prolonged initially and returned to baseline, platelet aggregation in PRP (ADP and thrombin) was ≥90% inhibited, and platelet aggregation in whole blood (thrombin) was inhibited initially and returned toward baseline over the 3 hr protocol (fig. 5A and B). These studies indicated that in dogs and monkeys, maximal inhibition of platelet aggregation induced by the weak platelet agonist, ADP, was required for in vivo antithrombotic efficacy. However, it was not necessary to maintain significantly prolonged bleeding time or inhibition of platelet aggregation induced by the potent platelet agonist, thrombin.

The dissociation of effects on bleeding time and inhibition of ex vivo platelet aggregation in animal models is consistent with results with the 7E3 antibody (Coller et al., 1989) but contrary to results with i.v. short-acting compounds (Ramjit et al., 1993; Lynch et al., 1995; Carteaux et al., 1993) in which plasma drug levels must be maintained for efficacy. The correlation of the more potent platelet agonists with the effect on bleeding time was previously demonstrated by Steiner et al. (1993) when it was found that inhibition of TRAP-induced platelet aggregation by lamifiban was more predictive of bleeding than inhibition of ADP-induced aggregation in human subjects. TRAP is a potent platelet agonist that mimics the effect of thrombin on platelets without generating fibrin (Connolly et al., 1994). It was also shown in a larger clinical trial in unstable angina patients with lamifiban that inhibition of TRAP-induced platelet aggregation, as compared to ADP-induced platelet aggregation, had a better correlation with bleeding time (Théroux et al., 1996). Although it appears that the effects of L-738,167 in dogs and monkeys and lamifiban in humans on bleeding time may be predicted by the effects of these compounds on thrombin- (or TRAP)-induced platelet aggregation, additional studies in humans would be required with each clinical compound to determine which parameter would be the best monitor of antiplatelet activity to provide effective and safe therapy.

Recent efforts have focused on the identification of orally active GPIIb/IIIa inhibitors for the chronic treatment of vasculocclusive disorders. Several compounds have shown enhanced oral bioavailability in preclinical animal studies (Müller et al., 1993; Nicholson et al., 1995; Szalony et al., 1995; Steiner et al., 1995; Weller et al., 1996) and in human trials (Simpfendorfer et al., 1996; Müller et al., 1995). This increase in oral bioavailability has been achieved by dosing with an inactive prodrug that requires metabolism for conversion to the active agent. An oral dose of the prodrug BIBU 104 of 10 mg/kg to rhesus monkeys significantly inhibited ex vivo platelet aggregation (83%); antiplatelet activity remained for 8 hr (44% inhibition) but platelet function had returned to pretreatment values at 24 hr (Müller et al., 1993). When the ester prodruk SC-54684A was administered orally to dogs and the active component, SC-54701A, was given i.v., the oral systemic availability of the active component, determined by bioassay, was estimated to be 21.3% (Nicholson et al., 1995). Further evaluation of SC-54684A in conscious dogs demonstrated that oral administration of high doses (2.4 mg/kg) twice daily achieved average inhibition of collagen-induced ex vivo platelet aggregation of 76 ± 3% (Szalony et al., 1995). A pilot clinical study with SC-54684A (xemlofiban) in unstable angina patients showed sustained inhibition of platelet aggregation with oral dosing three times daily (Simpfendorfer et al., 1996). It was also demonstrated that reasonable oral bioavailability could be achieved with a double prodruk, Ro 48-3657, which incorporates both a carboxylate ester and an amidoxime ester (Weller et al., 1996). Preclinical studies showed that oral administration of Ro 48-3657 resulted in 33 ± 6% bioavailability of the active derivative and the elimination half-life was 5.1 ± 1.4 hr in rhesus monkeys (Weller et al., 1996). Although the ultimate clinical dosing level and frequency must be determined in humans, preclinical and preliminary human studies suggest a minimum of twice daily oral dosing with these prodrugs.

Pharmacokinetic analysis of oral and i.v. administration of L-738,167 in dog showed low oral bioavailability (unpublished) and an extremely long plasma half-life (~96 hr) (Prueksaritanont et al., in press). Pharmacodynamic results of the present studies demonstrated that antplatelet activity after administration of low i.v. doses of L-738,167 was extended in dog (fig. 2), monkey and chimpanzee. In dog, very low oral doses were required for profound, sustained antiplatelet activity; a single oral dose of 25 μg/kg elicited 30 to 50% inhibition and the slightly higher oral dose of 100 μg/kg caused 100% inhibition of ADP-induced aggregation (fig. 6A). The antiplatelet activity elicited by 100 μg/kg (p.o., capsule) in dogs was initially 100%, then declined gradually and was maintained at more than 50% inhibition of aggregation for at least 72 hr after dosing (fig. 6B). In addition, it was shown that only one oral dose of L-738,167 per day to dogs and rhesus monkeys was required to maintain significant trough levels of antiplatelet activity (60–90% inhibition of ADP-induced aggregation, fig. 7). From this study and other investigations of this compound it appears that L-738,167 binds with high affinity to platelet GPIIb/IIIa (Bednar et al., in press), that the long plasma half-life is dictated by the slow separation of effects on platelet aggregation (ADP) and bleeding time. This allowed us to more precisely examine the prolongation of bleeding time that coincided with in vivo antithrombotic efficacy. Results showed that CFRs were inhibited by all doses of L-738,167 (3, 4 and 5 μg/kg i.v.) immediately after dosing and that the time course of the return of CFRs initiated at 1-hr intervals was dose related (fig. 4A). There was a separation of effects on platelet aggregation (ADP) and bleeding time, as described above; i.e., ADP-induced platelet aggregation remained inhibited and bleeding time returned toward baseline values over time. However, the effects on aggregation induced by the more potent platelet agonist, thrombin, in both PRP and whole blood decreased over time (fig. 5B). In addition to these findings in dog, a study was conducted in African green monkeys to determine if the relationship among effects on bleeding time, platelet aggregation and in vivo efficacy seen in dog was consistent in non-human primates. Results showed that L-738,167 (10 μg/kg i.v.) completely inhibited carotid artery CFRs, bleeding time was prolonged initially and returned to baseline, platelet aggregation in PRP (ADP and thrombin) was ≥90% inhibited, and platelet aggregation in whole blood (thrombin) was inhibited initially and returned toward baseline over the 3 hr protocol (fig. 5A and B). These studies indicated that in dogs and monkeys, maximal inhibition of platelet aggregation induced by the weak platelet agonist, ADP, was required for in vivo antithrombotic efficacy. However, it was not necessary to maintain significantly prolonged bleeding time or inhibition of platelet aggregation induced by the potent platelet agonist, thrombin.

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Recent efforts have focused on the identification of orally active GPIIb/IIIa inhibitors for the chronic treatment of vaso-
bound. Although, unlike prodrugs, the oral bioavailability of L-738,167 is inherently low, the potency and binding characteristics allow for low dose, once daily oral administration to maintain antithrombotic efficacy.

In conclusion, L-738,167 is a potent, orally active compound with long-duration antiplatelet activity suitable for once daily, low dose, oral administration in dogs and monkeys. It was efficacious in several canine models of arterial thrombosis andpotently prevented platelet-dependent CFPs in primates. In vivo efficacy was achieved in the absence of prolonged bleeding time; the potent platelet agonist, thrombin, was a better predictor of elevated bleeding times than the weak agonist, ADP. Therefore, L-738,167 possesses many desirable characteristics of a clinically useful oral GPIIb/IIIa antagonist for chronic therapy.

References


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