

Protective Effect of Oral Xemilofiban in Arterial Thrombosis in Dogs

Increased Activity in Combination With Aspirin

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Background—Inhibition of platelet aggregation by preventing the binding of fibrinogen to glycoprotein (GP) IIb/IIIa on activated platelets results in antithrombotic activity. We report on the antithrombotic effect of xemilofiban (SC-54684A), an oral GP IIb/IIIa antagonist, administered alone or with aspirin (ASA) in an acute thrombosis model.

Methods and Results—Conscious dogs were treated with xemilofiban (1.25, 2.5, 5.0, or 6 mg/kg, n=6); low-dose (LD, 81 mg) ASA, n=7; high-dose (HD, 162 mg) ASA, n=6; xemilofiban 1.25 mg/kg plus LD ASA, n=6; xemilofiban 1.25 mg/kg plus HD ASA, n=6; or placebo, n=7. Dogs were anesthetized 60 minutes later, and the effects of the treatments were evaluated after electrolytic injury (250 μ A for 180 minutes) in the left circumflex coronary artery. Bleeding time (BT) was assessed in a separate study. Incidence of thrombosis was reduced ($P<0.05$) by xemilofiban ≥ 2.5 mg/kg, HD ASA, or xemilofiban 1.25 mg/kg plus HD ASA compared with placebo. Xemilofiban ≥ 2.5 mg/kg or xemilofiban 1.25 mg/kg plus HD ASA significantly increased time to occlusion, inhibited ex vivo platelet aggregation to collagen $>90\%$, and prevented or decreased ($P<0.05$) cyclic flow variations (CFVs) compared with placebo. BT was increased ($P<0.05$) with xemilofiban ≥ 2.5 mg/kg but not with xemilofiban 1.25 mg/kg plus HD ASA.

Conclusions—Xemilofiban ≥ 2.5 mg/kg, HD ASA, or xemilofiban 1.25 mg/kg plus HD ASA significantly reduced the incidence of thrombosis. These doses of xemilofiban or xemilofiban 1.25 mg/kg plus HD ASA increased time to occlusion, inhibited ex vivo platelet aggregation by $>90\%$, and prevented or reduced CFVs. Xemilofiban ≥ 2.5 mg/kg but not xemilofiban 1.25 mg/kg plus HD ASA significantly increased BT. (*Circulation*. 1998;98:813-820.)

Key Words: platelet aggregation inhibitors ■ glycoproteins ■ thrombosis

The binding of adhesion protein ligands, such as fibrinogen and von Willebrand factor, to activated platelets has been identified as the final step in platelet aggregation, and this binding is completely mediated by GP IIb/IIIa.¹ There is abundant evidence that the formation of platelet aggregates plays an essential role in the pathogenesis of acute arterial thrombosis and is associated with such vascular diseases as unstable angina, transient ischemic attack, and myocardial infarction.² Thus, effective inhibition of platelet activity and platelet aggregation is a primary target for the development of drugs designed to treat various cardiovascular diseases. Consequently, the continual search for and development of antithrombotic agents that block fibrinogen-GP IIb/IIIa binding on activated platelets is a major goal of cardiovascular research.

Xemilofiban (SC-54684A; Figure 1) is an orally active antithrombotic agent that is currently in clinical trials. After administration, xemilofiban is rapidly metabolized to SC-54701. The hydrochloride salt (SC-54701A) of the active moiety of xemilofiban is a potent inhibitor of fibrinogen binding to platelets.³ This compound is highly selective for the GP IIb/IIIa receptor compared with other integrins shar-

ing the same β_3 -subunit.³ ASA is the most convenient and widely tested antiplatelet agent, but this drug possesses limited antithrombotic efficacy. Clinical studies show that the incidence of acute myocardial infarction is reduced 40% by ASA,⁴ yet the event may occur during ASA therapy in 1% of individuals free of prior myocardial infarction,⁵ in 4% of patients with unstable angina,⁶ and in 24% of patients with prior myocardial infarction.⁷ After successful thrombolytic therapy, rethrombosis may occur in patients even though the treatment regimen includes oral ASA and anticoagulation with heparin.⁸ Thus, there is a need for newer orally active agents with antiplatelet and antithrombotic properties that would add to the therapeutic armamentarium for thrombosis-related disorders. Previously, we have shown that intravenous ASA and concomitant administration of heparin reduce the dose of intravenous SC-54701A that provides antithrombotic efficacy in a canine model of occlusive coronary artery thrombosis.⁹

Initial animal studies with xemilofiban demonstrated its effectiveness as an antiplatelet agent.¹⁰ Accordingly, the present investigation determines the antithrombotic activity of orally administered xemilofiban in a canine model of occlusive coro-

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Selected Abbreviations and Acronyms

ASA	= aspirin
BT	= bleeding time
CFV	= cyclic flow variation
GP	= glycoprotein
HD	= high-dose
LCx	= left circumflex coronary artery
LD	= low-dose
PPP	= platelet-poor plasma
PRP	= platelet-rich plasma
TX	= thromboxane

nary artery thrombosis⁹ and tests the hypothesis that targeted levels of inhibition of platelet aggregation by xenilofiban prevent thrombotic occlusion. Moreover, the present experiments determine the antithrombotic effect of a minimally effective dose of xenilofiban combined with oral ASA.

Methods

Reagents

Xenilofiban was synthesized at Searle. Xenilofiban was administered as the hydrochloride salt (SC-54684A), and the doses were calculated on the basis of the free base (SC-54684, MW=358.4). A 1-mg/kg dose of the ester is 2.79 $\mu\text{mol/kg}$, which is equivalent to 1.10 mg/kg of hydrochloride salt. SC-54701B, the trifluoroacetate salt of the active moiety, was used in the *in vitro* experiments. Concentrations of SC-54701B were determined on the basis of the free base (SC-54701, MW=330.3). Generic oral ASA was purchased locally, and soluble lysine ASA was obtained from Synthelabo. Collagen from equine tendon was purchased from Chronolog Corp. ADP, U46619 (a thromboxane mimic), and sodium citrate were purchased from Sigma Chemical Co. Arachidonic acid and epinephrine were obtained from Bio/Data Corp. TXB₂, used as an indirect measurement of TXA₂ production, was measured by the Bioanalytical Laboratory at Searle by use of a TXB₂ Enzyme Immunoassay Kit obtained from Cayman Chemical Co.

Experimental Procedures

A schematic of the protocol for the experiments performed in this study is presented in Figure 2.

Oral Drug Administration

Male or female mongrel dogs (14 to 27 kg) were preselected on the basis of general health and a platelet count of $\approx 200 \times 10^3$ to $250 \times 10^3/\mu\text{L}$. The dogs were fasted for 12 to 18 hours before the start of testing and were divided into the following treatment groups: xenilofiban (single dose of 1.25, 2.5, 5.0, or 6 mg/kg, $n=6$ /group); LD (81 mg) ASA, $n=7$; HD (162 mg) ASA, $n=6$; xenilofiban 1.25 mg/kg plus LD ASA, $n=6$; and xenilofiban 1.25 mg/kg plus HD ASA, $n=6$. Xenilofiban or placebo (sucrose, $n=7$) was adminis-

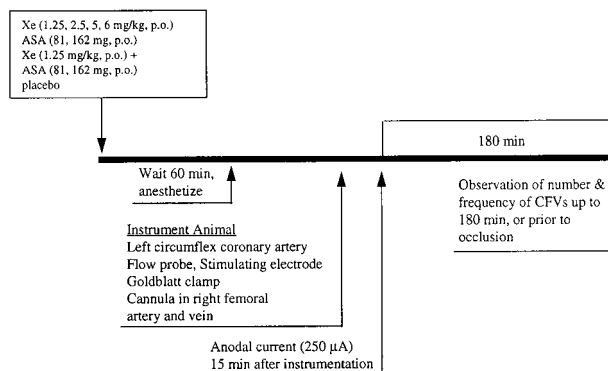


Figure 2. Schematic of experimental protocol used in study. Xenilofiban (Xe), ASA, Xe plus ASA, or placebo ($n=6$ or 7 per group) was administered before electrolytic induction of thrombosis in LCx. Incidence of occlusive thrombus formation, time to arterial occlusion, and inhibition of *ex vivo* platelet aggregation were monitored.

tered to conscious dogs by soft gelatin capsules. Throughout this article, the dose of xenilofiban is 1.25 mg/kg when used in combination with LD ASA or HD ASA.

Surgical Procedures

One hour after treatment, the experimental model was used essentially as previously described.⁹ Briefly, the dogs were anesthetized with sodium pentobarbital (30 mg/kg IV, supplemented as needed) and placed on a respiratory pump (Biological Research Apparatus). The right femoral artery and vein were cannulated for monitoring arterial blood pressure (Micron Instruments) and for withdrawing blood samples, respectively. The heart was exposed, and the LCx was isolated. The artery was instrumented with a Doppler flow probe for monitoring blood flow velocity (Crystal Biotech), a stimulation electrode, and a Goldblatt clamp. The clamp was adjusted to reduce the reactive hyperemic response without affecting resting LCx blood flow. The stimulation electrode was connected in series with a 12- to 112-k Ω variable resistor to the positive terminal of a 9-V battery. The negative terminal of the battery was connected to an electrode placed in a subcutaneous site. Thrombotic occlusion of the LCx was initiated ≈ 15 minutes after surgical preparation by application and maintenance of 250 μA of continuous current to the stimulation electrode. The current lasted for 180 minutes unless the dog died after LCx occlusion. Thrombotic occlusion occurred when the LCx blood flow decreased to and remained at zero for a minimum of 30 minutes. During electrical stimulation, before an occlusive thrombus was formed, the number and frequency (No./min) of CFVs were recorded. The frequency of CFVs in coronary blood flow preceding the formation of an occlusive thrombus was expressed as $\times 100$ because of the small values obtained. At the end of the experiment, the dogs were euthanized with an overdose of sodium pentobarbital. Light microscopy was used to assess mural thrombosis at the site of injury in the LCx of selected dogs treated with xenilofiban. The section of the LCx chosen for this procedure had no observable thrombus in the lumen of the vessel.

Ex Vivo Platelet Aggregation and Platelet Counts

Before treatment, blood was drawn from the cephalic vein of the dogs to determine platelet counts (S-Plus IV cell counter, Coulter) and *ex vivo* collagen-induced platelet aggregation. Blood used for platelet counts was collected into evacuated tubes containing 7.5% EDTA, and blood for platelet aggregation was collected into evacuated tubes containing 3.8% sodium citrate.⁹ Assays for the determination of inhibition of platelet aggregation were repeated 1 hour after treatment while the dogs were conscious and at 1-hour intervals while the dogs were anesthetized. The blood was centrifuged for 6 minutes at 266g (Sorvall Technospin R centrifuge; H-5094 rotor) at room temperature ($\approx 24^\circ\text{C}$), and PRP was removed. The remaining blood was centrifuged for 10 minutes at 2000g at room temperature,

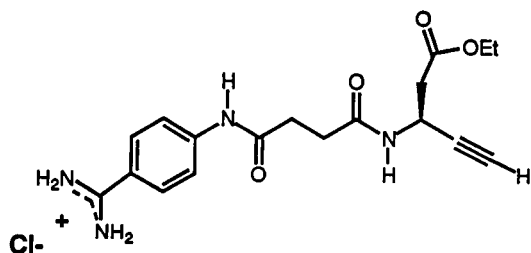


Figure 1. Chemical structure of xenilofiban: ethyl 3S-[[4[[4(aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentynoate monohydrochloride. Xenilofiban is also known as SC-54684A.

and PPP was removed. Samples were assayed on a PAP-4 platelet aggregometer (Bio/Data Corp) with PPP as the blank. Platelet aggregation was performed by adding 50 μ L of collagen (33 or 40 μ g/mL final concentration) to 450 μ L of PRP and measuring aggregation for 3 minutes. (The 2 concentrations of collagen were used to produce maximum aggregation with different lots of collagen.) Collagen was used because it provided a better relationship between protection and inhibition than ADP in this model.

Bioassay for Plasma Levels of the Active Moiety of Xenilofiban

The procedures of the bioassay were described previously.⁹ Briefly, the bioassay used plasma from treated dogs as the source of inhibitor to be tested in vitro against normal (naive) platelets from donor dogs. PRP from nontreated dogs was added to wells containing plasma samples from treated dogs in a 96-well microtiter plate. ADP (20 μ mol/L) was added to the platelet suspension in each well to induce aggregation. Optical density at 405 nm was measured on all wells simultaneously in a plate reader (Molecular Devices). The results were quantified by comparison to a standard inhibition curve prepared in plasma with the use of known amounts of SC-54701A.

Template BT

To assess BT with the treatments that prevented occlusive thrombus formation, a separate study was conducted in a separate group of beagle dogs. Xenilofiban (2.5 or 6 mg/kg, n=4 per treatment) or xenilofiban plus HD ASA (n=4) was administered to fasted, conscious dogs as described above. Sixty minutes after treatment, the dogs were anesthetized with sodium pentobarbital (30 mg/kg IV, supplemented as needed). The effect of treatment on upper-lip BT was observed after an additional 120 minutes (comparable to the time allowed for treatment and surgery with the other dogs in the main study). BT was measured with a Simplate IIR device (Organon Teknika Corp). Duplicate tests were performed on each dog.

In Vitro Effect of Xenilofiban+ASA

In a separate study, PRP and PPP were prepared as previously described. PRP (400 μ L) was added to cuvettes containing 50 ng/mL SC-54701B with or without ASA (100 μ g/mL) or ASA alone. The choice of the concentration of SC-54701B was based on plasma concentrations measured at the time of arterial occlusion or at the end of the experiment (see Table 2, xenilofiban 1.25 mg/kg). The concentration of ASA used was based on calculations using HD ASA, average dog body weight, and estimated plasma volume of the dogs. Controls without SC-54701B or ASA were also carried out. After the addition of the agonist, platelet aggregation was monitored for 2 minutes with ADP (20 μ mol/L) or arachidonic acid (0.2 mg/mL) and for 3 minutes with collagen (40 μ mol/L) or U46619 (100 μ mol/L). [Maximal aggregation was achieved with 100 μ mol/L U46619 after priming with epinephrine (60 μ mol/L).] The contents of the cuvette were transferred to Eppendorf centrifuge tubes containing 10 μ L of ASA to prevent further TXA₂ production, and the tubes were centrifuged at 12 000g for 3 minutes. Supernatants were aspirated and frozen for determination of TXB₂ levels.

Data Analysis

Unless otherwise stated, values are expressed as mean \pm SEM. The 1-tailed χ^2 trend test was applied to the rank-transformed data. Tukey's pairwise test (2-tailed) was used for additional comparisons. The Mantel-Haenszel test was applied only for the response "incidence of occlusive thrombus," which is by its nature a discrete valued variable. An independent *t* test was used to compare BT. A value of *P*<0.05 was considered to be statistically significant.

Results

Group Characteristics

Platelet counts and platelet aggregation were not statistically different among the 9 groups of dogs before treatment.

TABLE 1. Incidence of LCx Occlusive Thrombus Formation and Mean CFV

Treatment	n	Occlusion, n (%)	Mean CFV/min, $\times 100$	Number of Dogs With CFV
Xenilofiban, mg/kg				
1.25	6	5/6 (83)	2.5 \pm 1.2	4/6
2.5	6	0/6 (0)*	0.0 \pm 0.0*	0/6
5	6	1/6 (17)*	0.8 \pm 0.8*	1/6
6	6	0/6 (0)*	0.0 \pm 0.0*	0/6
ASA, mg				
LD, 81	7	6/7 (86)	2.6 \pm 1.0	5/7
HD, 162	6	3/6 (50)*	0.8 \pm 0.3	4/6
Xenilofiban, 1.25 mg/kg				
+LD ASA	6	3/6 (50)	0.7 \pm 0.3	3/6
+HD ASA	6	0/6 (0)*	0.0 \pm 0.0*	0/6
Placebo	7	7/7 (100)	2.0 \pm 0.6†	5/6†

CFV values are mean \pm SEM.

**P*<0.05 vs placebo.

†Missing CFV data from 1 dog due to technical problems.

Ventricular fibrillation occurred after complete thrombotic occlusion in a dog treated with xenilofiban plus LD ASA. This dog was not excluded from data analysis.

Prevention of Occlusive Thrombus

The effects of xenilofiban, ASA, or both on incidence of LCx occlusion are shown in Table 1. Incidence of occlusion was significantly reduced by 2.5, 5, or 6 mg/kg of xenilofiban. The LCx remained patent in all dogs receiving 2.5 or 6.0 mg/kg of xenilofiban and in 5 of 6 dogs treated with 5 mg/kg of the compound. The LCx remained patent in only 1 dog treated with 1.25 mg/kg of xenilofiban and 3 of 6 dogs treated with HD ASA. When the low dose of xenilofiban was combined with HD ASA, no occlusive thrombus was formed during the entire 180-minute study period. Typically, patent vessels, as expected, showed disruption of the endothelial surface and discontinuities in the internal elastic lamina (Figure 3). However, other than occasional margined neutrophils, platelets, and red blood cells, the lumens were clear. All dogs treated with placebo developed complete thrombotic occlusion. Figure 4 shows a schematic of the patency status of the LCx for selected treatment groups.



Figure 3. Patent section of coronary vessel after electrical endothelial damage. Endothelium and portions of internal elastic lamina have been lost. On this surface are margined neutrophils and red blood cells but very few platelets or fibrin strands. Notwithstanding these occasional accumulations of cells along damaged vessel wall, this artery was patent at time of fixation. Toluidine blue-stained 1- μ m section, $\times 480$. Lumen is at top of figure.

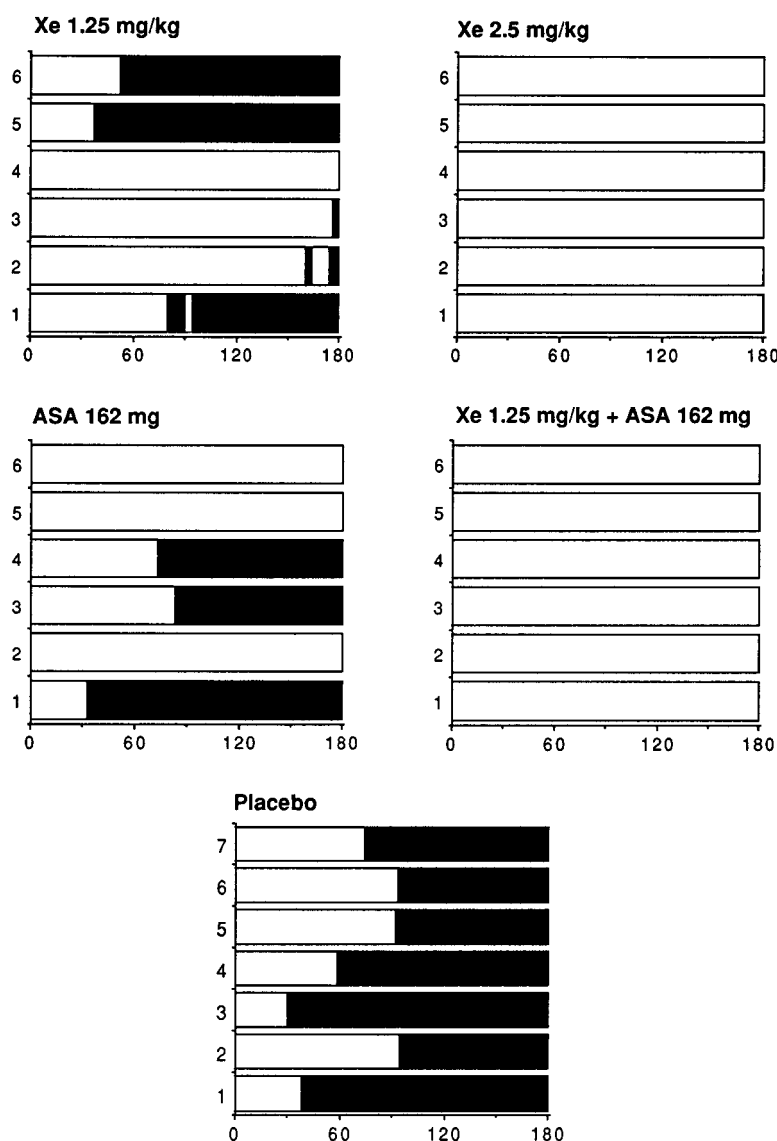


Figure 4. Schematic of LCx patency status in dogs treated orally with selected doses of xenilofiban (Xe), Xe plus ASA, or placebo control. Each horizontal bar represents response of 1 dog to 180 minutes of continuous anodal current. The current is started approximately 3 hours after treatment. The open portions represent periods of measurable blood flow continuously monitored by a pulsed Doppler system, and the shaded portions represent periods of LCx occlusion.

Figure 5A shows the effect of the treatments on inhibition of platelet aggregation at the time of complete occlusion or at the end of the experiment (if LCx occlusion did not occur). Inhibition of platelet aggregation was significantly increased by xenilofiban (1.25 mg/kg, $59 \pm 15\%$; 2.5 mg/kg, $94 \pm 4\%$; 5 mg/kg, $98 \pm 2\%$; 6 mg/kg, $98 \pm 2\%$), ASA (LD, $30 \pm 12\%$; HD, $78 \pm 9\%$), and the combined treatment (xenilofiban+LD ASA, $95 \pm 4\%$; xenilofiban+HD ASA, $99 \pm 0\%$) compared with placebo ($2 \pm 1\%$). Notably, platelet aggregation was inhibited to a greater extent with xenilofiban plus HD ASA than when either treatment was used alone. Inhibition of aggregation by the 2 highest doses of xenilofiban or the combined treatment was significantly greater than that of the 1.25 mg/kg dose of xenilofiban. All treatments, except 1.25 mg/kg of xenilofiban or HD ASA, significantly increased inhibition of platelet aggregation compared with LD ASA. Only 5 mg/kg of xenilofiban produced a statistically significant increase in the inhibition level relative to HD ASA.

The effect of the treatments on time to zero flow in the LCx is shown in Figure 5B. Dogs with a patent LCx at the end of

the experiment were assigned a value of >180 minutes (censored) for time to zero flow, and 180 minutes was used for comparative purposes. The LCx of placebo dogs occluded in 68 ± 10 minutes. Compared with placebo, significant prolongation of time to zero flow was noted in the dogs treated with xenilofiban (2.5 mg/kg, >180 minutes; 5 mg/kg, 160 ± 20 minutes; 6 mg/kg, >180 minutes) and in the group of dogs receiving the combined treatment (xenilofiban+LD ASA, 139 ± 25 minutes; xenilofiban+HD ASA, >180 minutes).

CFVs During Anodal Stimulation of the LCx

A summary of the mean CFVs observed during stimulation of the LCx is also presented in Table 1. Five of 6 dogs treated with placebo had CFVs; the data on CFVs were not obtained from the remaining dog because of technical problems. CFVs were not observed with dogs treated with either 2.5 or 6 mg/kg xenilofiban. The CFV observed with 5 mg/kg xenilofiban was attributed to the dog that had a thrombotic occlusion (see Table 1 and "Plasma Levels of the Active

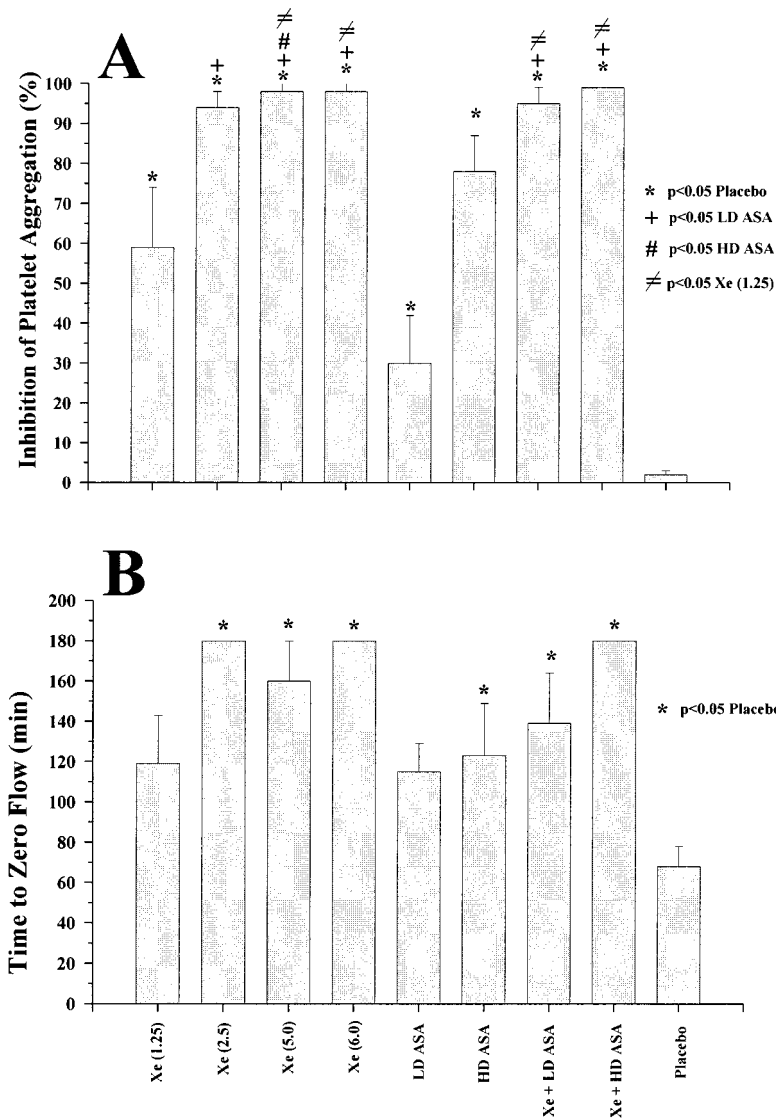


Figure 5. Effects of oral administration of xemilofiban (Xe), ASA, Xe plus ASA, or placebo on inhibition of ex vivo collagen-induced platelet aggregation (A) and on corresponding time to occlusion of LCx (B). Time to zero flow of 180 minutes represents maximum time of current application with no thrombotic occlusion. n=6 or 7 per treatment group. Values are mean±SEM. *P<0.05 vs placebo; +P<0.05 vs LD ASA; #P<0.05 vs HD ASA; ≠P<0.05 vs Xe 1.25 mg/kg.

Moiety" below). Neither ASA alone nor xemilofiban plus LD ASA significantly reduced CFVs compared with placebo. No CFVs were observed with the treatment of xemilofiban plus HD ASA.

Plasma Levels of the Active Moiety

Table 2 shows that the active moiety was increased in a dose-dependent manner at the time of thrombotic artery occlusion or at the end of the experiment. In general, protection against occlusive thrombus formation was achieved in all dogs when levels of the active moiety were >100 ng/mL (Figure 6). The dog with the occlusive thrombus and the CFVs after treatment with 5 mg/kg xemilofiban had a maximum xemilofiban plasma level of 31 ng/mL.

Template BT

BT was assessed in duplicate in dogs treated with 2.5 or 6 mg/kg of xemilofiban and dogs treated with xemilofiban plus HD ASA (Table 3). Both doses of xemilofiban significantly prolonged BT relative to baseline. However,

the increase in BT by the lower dose of xemilofiban (the lowest dose that provided antithrombotic protection in all dogs in the main study) was modest. Three of the 4 dogs treated with 6 mg/kg of xemilofiban exhibited a BT of >30

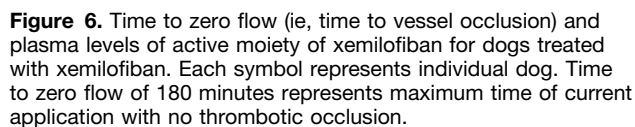
TABLE 2. Plasma Levels of the Active Moiety of Xemilofiban With the Related Ex Vivo Inhibition of Collagen-Induced Platelet Aggregation

Xemilofiban, mg/kg	Plasma Level,* ng/mL	Percent Inhibition*
1.25	37±6†	59±15
2.5	109±32	94±4
5	221±45	98±2
6	361±54‡	98±2

*Represents plasma level and percent inhibition at the time of arterial occlusion or at the end of experimentation, if occlusion did not occur.

†One of the 6 dogs had a plasma level of xemilofiban that was below the minimum sensitivity (20 ng/mL) of the bioassay, and therefore, 20 ng/mL was used.

‡At the end of testing, 1 of the 6 dogs had a plasma level of xemilofiban that exceeded the maximum sensitivity (600 ng/mL) of the bioassay, and therefore, 600 ng/mL was used. Values are mean±SEM (n=6 per treatment).



Mechanism of Increased Activity of Xemilofiban With ASA

Discussion

TABLE 3. Effect of Xemilofiban Alone or in Combination With ASA on Mean Template BT

Values are mean \pm SEM (n=4 per treatment).
* $P < 0.05$ vs baseline.

The efficacy of ASA as an antiplatelet agent has been extensively investigated, and it remains the most widely used and cost-effective drug in the prevention of platelet aggregation.²⁰ ASA, however, is not universally effective at inhibiting platelet-dependent coronary artery thrombosis, because non-cyclooxygenase-dependent platelet aggregation commonly occurs *in vivo*, and ASA activity is limited to inhibition of the cyclooxygenase pathway.²¹ The finding that ASA (the terms “low” and “high” doses of ASA are used here to compare the 2 doses with each other and are not meant to refer to treatment levels of ASA in either dogs or humans) was essentially ineffective in preventing thrombosis in the LCx was expected, because we⁹ and others²² using this arterial thrombosis

TABLE 4. TXA₂ Production and Platelet Aggregation with SC-54701B, ASA, SC-54701B in Combination With ASA or Control

		ADP	Collagen	Arachidonic Acid	U46619
		20 μ mol/L	40 (μ g/mL)	0.2 mg/mL	100 μ mol/L
TXA ₂ , ng/ml	Control	19	368	804	11
	ASA	0.12	0.25	1.61	0.64
	SC-54701B	2.21	72	790	1.67
	SC-54701B+ASA	0.21	0.19	2.09	0.60
% Aggregation	Control	75	87	19	74
	ASA	41	11	2	77
	SC-54701B	0	2	0	0
	SC-54701B+ASA	0	0	1	0

Data were generated from duplicate tests for each treatment.

model have previously shown that the effects of intravenous ASA were limited in the prevention of arterial thrombosis. This is probably because ASA works by inhibiting cyclooxygenase and therefore blocks the synthesis of the endoperoxides, prostaglandins, and thromboxanes. ASA will not eliminate all possible proaggregatory mechanisms, whereas inhibition of fibrinogen binding to platelets represents the final common pathway and blocks all platelet aggregation and thrombus formation. The ineffectiveness of ASA as an antithrombotic agent has also been reported in other canine models.^{23,24}

Because patients requiring antithrombotic therapy often take ASA as adjunctive treatment, we tested the effect of low-dose xemilofiban in combination with oral ASA. Treatment with 1.25 mg/kg of xemilofiban or HD ASA prevented LCx occlusion in $\leq 50\%$ of the dogs. When both treatments were combined, the observed antithrombotic effect was increased. Because the mechanisms of the platelet-inhibitory effect of xemilofiban and ASA differ, and each agent inhibited platelet aggregation to some extent, one might expect that the combination of a suboptimal dose of xemilofiban and ASA would result in an increased antiaggregatory response. Increased antiaggregatory activity from the administration of a GP IIb/IIIa receptor antagonist combined with ASA has been reported by others.²⁵ In the *in vitro* mechanism study, SC-54701B inhibited TXA₂ (a potent agonist of platelet aggregation) production in a dose-related manner. When a suboptimal dose of SC-54701B was combined with ASA, increased inhibition of TXA₂ was observed. The ability of xemilofiban to prevent platelet aggregation, resulting in an indirect decrease of TXA₂, coupled with the additional inhibition of aggregation and TXA₂ release by ASA, probably contributed substantially to the mechanism by which high-dose ASA potentiated the effect of 1.25 mg/kg of xemilofiban in dogs.

Although BT was significantly increased by xemilofiban in the present study, BT does not predict surgical bleeding.²⁶ (BT was modestly lengthened by 2.5 mg/kg of xemilofiban, the minimum antithrombotic dose. A further prolongation of BT was observed with 6 mg/kg of xemilofiban.) Results from clinical trials have demonstrated that GP IIb/IIIa blockade reduced ischemic/thrombotic events after coronary interven-

tion, but at the cost of an increased risk of bleeding complications.²⁷ This increased risk was partly due to the concomitant administration of heparin. Concerns about excessive bleeding in the setting of treatment with a GP IIb/IIIa receptor antagonist (with ASA and heparin) have been allayed by careful titration of heparin along with prudent vascular access site management.²⁸ Ticlopidine is an example of an antiplatelet drug that prolongs BT without increasing operative blood loss.²⁹ Results from our main study showed that a suboptimal dose of xemilofiban plus ASA did not significantly alter BT, suggesting that administration of xemilofiban with ASA may be used in the clinic for prevention of acute thrombotic events without impairment of hemostasis.

Unlike the 67% (4 of 6) of the dogs with CFVs after treatment with the 1.25 mg/kg dose of xemilofiban or HD ASA, the combination of the two treatments provided increased protection against CFVs. This effect was similar to that produced by the 2.5- or 6-mg/kg dose of xemilofiban. The mechanism of CFVs has been associated with the release of such vasoactive substances as serotonin and TXA₂³⁰ and with periodic acute occlusive platelet thrombus formation followed by embolization.³¹

Orally active compounds are expected to be more readily employed for preventive antithrombotic therapy. Xemilofiban has the potential to meet this goal. The degree of platelet inhibition necessary for this agent to be efficacious in the patient population is currently being assessed in a variety of clinical trials. Sustained inhibition of platelet aggregation with xemilofiban has been demonstrated in patients with unstable angina³² and in patients after coronary stent deployment.³³

In summary, xemilofiban, a novel GP IIb/IIIa receptor antagonist, inhibits *ex vivo* platelet aggregation, prevents occlusive thrombus formation, and prevents CFVs in response to continuous electrical injury in a canine model of arterial thrombosis. In addition, coadministration of HD ASA provides an observed increase in the antithrombotic response and in the level of protection against CFVs by a dose of xemilofiban that when administered alone has limited efficacy in this model.

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