British Journal of Pharmacology (2002) 136, 927–937

www.nature.com/bjp

Prevention of experimental carotid and coronary artery thrombosis by the glycoprotein IIb/IIIa receptor antagonist CRL42796

[1]James K. Hennan, [1]Ting-Ting Hong, [1]David E. Willens, [1]Edward M. Driscoll, [2]Thierry A. Giboulot & *,[1]Benedict R. Lucchesi

[1]Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan, MI 48109-0632, U.S.A. and [2]Centre de Recherches du Laboratoire L. Lafon, 19 Avenue du Professeur Cadiot, 94700 Maisons Alfort, France

1 The antithrombotic effect of the glycoprotein IIb/IIIa receptor antagonist, CRL42796, was examined in canine models of carotid and coronary artery thrombosis.

2 In the carotid artery thrombosis model, occlusion occurred in all control vessels (time to thrombosis 47.6 ± 8.9 min). After treatment with low dose CRL42796 (15 μg kg^{-1} loading dose $+0.31$ μg kg^{-1} min^{-1} i.v.), two of five vessels occluded. Time to thrombosis increased significantly to 155.2 ± 23.1 min. When the drug infusion was increased (0.69 μg kg^{-1} min^{-1}), each of five vessels remained patent.

3 *Ex vivo* platelet aggregation in response to arachidonic acid (AA) and ADP was examined in platelet rich plasma (PRP) prepared from citrate or heparin anticoagulated blood. CRL42796 reduced platelet reactivity at low and high doses in PRP from citrate anticoagulated blood. However, in PRP from heparin anticoagulated blood, only the higher infusion dose produced a significant reduction in *ex vivo* platelet responses.

4 A combination of oral aspirin (4.6 mg kg^{-1} -41, -17 h) and the low infusion dose of CRL42796 did not produce an additional benefit beyond that provided by CRL42796 alone.

5 Coronary artery thrombosis was inhibited in four of five vessels treated with the lower infusion dose of CRL42796 and in five of five vessels treated with the higher infusion. Time to thrombosis increased with both doses (Control, 90.8 ± 10.4 min; low dose, 165.8 ± 14.2 min; high dose, $>180.0 \pm 0$ min).

6 The results indicate that CRL42796 is an effective *in vivo* antithrombotic agent against experimentally-induced carotid and coronary artery thrombosis.
British Journal of Pharmacology (2002) **136,** 927–937

Keywords: Arterial thrombosis; GPIIb/IIIa receptor antagonist; CRL47926; aspirin; platelet aggregation; arachidonic acid; bleeding time; vessel wall injury; arterial stenosis.

Abbreviations: AA, arachidonic acid; COX-1, cyclo-oxygenase-1; COX-2, cyclo-oxygenase-2; LCX, left circumflex; PPP, platelet poor plasma; PRP, platelet rich plasma

Introduction

Accepted approaches to the management of patients presenting with an evolving myocardial infarction involve the use of angioplasty or thrombolytic therapy (streptokinase, activase, retavase, etc.) coadministered with both heparin and acetylsalicylic acid (aspirin) (Gensini *et al*., 1999; Gibson, 1999; Spinler *et al*., 2001). Whereas either angioplasty or administration of a thrombolytic agent is effective in restoring arterial blood flow, maintaining vessel patency depends upon adjunctive therapy directed at inhibition of thrombin generation and/or platelet reactivity. Several antiplatelet agents (abciximab, eptifibatide, tirofiban, ticlopidine, clopidogrel), having mechanisms of action differing from that of aspirin, show efficacy in reducing the incidence of thrombotic occlusion after restoration of arterial blood flow (Spinler *et al*., 2001).

Platelets serve an important role in arterial thrombosis and represent a predominant pharmacologic target for the prevention of reocclusion after successful recanalization of an occluded vessel. Although aspirin can modulate platelet reactivity, its antiplatelet potential is achieved as a result of inhibition of cyclo-oxygenase, essential for conversion of arachidonic acid to thromboxane A_2. The latter serves as a platelet activator and arterial vasoconstrictor. Aspirin is effective in preventing *ex vivo* arachidonic acid-induced platelet aggregation, but lacks significant antiaggregatory efficacy against other platelet agonists (5-HT, epinephrine, thrombin, plasmin, etc.). On the other hand, pharmacologic interventions directed against the platelet glycoprotein (GP) IIb/IIIa receptor are effective antiaggregatory agents regardless of the inciting agonist (Makkar *et al*., 1997).

The present study was designed to evaluate a new platelet GPIIb/IIIa receptor antagonist, CRL42796, for its anti-

*Author for correspondence at: Department of Pharmacology, University of Michigan Medical School, 1301C Medical Science Research Building III, Ann Arbor, Michigan, MI 48109-0632, U.S.A.; E-mail: benluc@umich.edu

thrombotic efficacy. Studies also were designed to explore the minimal effective dose of CRL42796 in combination with aspirin. Anaesthetized canines were subjected to electrolytic injury of the carotid or coronary arteries for induction of primary arterial thrombosis. The latter is defined as an occlusive thrombus secondary to arterial wall injury. Previous studies in our laboratory demonstrated that GPIIb/IIIa receptor antagonists are effective in preventing primary thrombus formation and maintaining vessel patency (Mickelson *et al*., 1990; Rote *et al*., 1994a). *Ex vivo* platelet aggregation and bleeding times were measured. The results demonstrate that CRL42796 is an effective antithrombotic agent with a minimal tendency to induce significant uncontrolled bleeding.

Methods

Guidelines for the use and care of experimental animals

The procedures used in this study are in accordance with the guidelines of the University of Michigan University Committee on the Use and Care of Animals and conform to the standards in The Guide for Care and Use of Laboratory Animals (NIH no. 86–23). The University of Michigan Unit for Laboratory Animal Medicine provided veterinary care.

Surgical preparation and model of vessel occlusion

Purpose-bred hound dogs, weighing 9–12 kg, were anaesthetized with sodium pentobarbital (30 mg kg^{-1}, i.v.). The animals were intubated and ventilated with room air using a Harvard respirator (Harvard Apparatus, Inc., Holliston, MA, U.S.A.), adjusted to deliver a tidal volume of 30 ml kg^{-1} at a frequency of 12 breaths min^{-1}. A catheter was inserted into the right and left femoral veins for drug administration and blood sampling. Arterial blood pressure was recorded from the right femoral artery using a Millar™ Mikro-tip catheter (Millar Instruments, Inc., Houston, TX, U.S.A.). A standard limb lead II electrocardiograph was recorded continuously to monitor heart rate. A calibrated ultrasonic flow probe (Model 2RB907, Transonic Systems Inc., Ithaca, NY, U.S.A.) was placed on the carotid artery and blood flow was monitored continuously. All physiologic parameters were recorded on a Grass polygraph interfaced to a MacLab (AD Instruments Pty Ltd, Castle Hill, Australia) data acquisition system and Macintosh™ computer (Apple Computer, Inc., Cupertino, CA, U.S.A.).

An intravascular electrode was inserted through the wall of the carotid artery and positioned so that the non-insulated intraluminal portion of the electrode was in firm contact with the intimal surface of the vessel. An external adjustable constrictor was applied to the artery. The intravascular electrode and mechanical constrictor were positioned distal with respect to the flow probe. The mechanical constrictor on the carotid artery was constructed of stainless steel, shaped to fit around the vessel. A nylon screw (2 mm in diameter) threaded through the C-shaped metal band was adjusted to decrease the circumference of the vessel thereby forming a regional stenosis. The vessel was narrowed to a point where the pulsatile flow pattern was reduced by 50% without altering the mean carotid artery blood flow.

Electrolytic injury to the intimal surface of the carotid artery was accomplished with the use of an intravascular electrode composed of a Teflon-insulated, silver-coated, copper wire. Penetration of the vessel wall by the electrode was facilitated by attaching the tip of a 25-gauge hypodermic needle to the distal, non-insulated part of the electrode. The intravascular electrode was connected to the positive pole (anode) of a dual-channel square wave generator (Grass S88 stimulator and a Grass Constant Current Unit, Model CCU1A). The cathode was connected to a distant subcutaneous site. The current delivered to the vessel was monitored continuously on an ammeter and maintained at 300 μA for induction of carotid artery vessel wall injury, and 150 μA when used to induce deep vessel wall injury in the coronary artery. The anodal electrode was positioned to have the non-insulated portion (3–4 mm) in intimate contact with the endothelial surface of the vessel. Proper positioning of the electrode in the vessel and evidence of vessel wall injury was confirmed by visual inspection at the conclusion of each experiment. The presence of the external constrictor results in localized turbulent flow and mimics the presence of an atheromatous plaque in a diseased vessel. The combined presence of deep vessel wall injury and turbulent flow leads to the progression of platelet-dependent occlusive thrombus formation. The anodal current was terminated 30 min after arterial occlusion as indicated by the absence of blood flow. On the other hand, the anodal injury current was maintained for a maximum duration of 3 h if the vessel remained patent as evidenced by the presence of blood flow after which the anodal current was suspended. In the latter instance, a value of 180 min was used as the 'time to occlusive thrombosis' for the purpose of statistical analysis.

Model of coronary artery thrombosis

The heart was exposed through a left thoracotomy at the sixth intercostal space and suspended in a pericardial cradle. A 1-cm segment of the left circumflex (LCX) coronary artery was exposed proximal to the first obtuse marginal branch and instrumented with a Transonic™ ultrasonic flow probe (Model 1.5RB, Transonic Systems Inc., Ithaca, NY, U.S.A.) for continuous monitoring of phasic coronary artery blood flow. Instrumentation of the canine coronary artery for induction of arterial thrombosis was similar to that described for the carotid artery. A ligature stenosis was placed around the LCX coronary artery such that the hyperemic response to a 10-s occlusion was reduced by 30%. Mean coronary artery blood flow was not affected by the stenosis. An intracoronary electrode was inserted through the LCX arterial wall so that the uninsulated portion was positioned against the endothelial surface. Deep vessel wall injury and occlusive arterial thrombosis was initiated *via* application of an anodal current (150 μA) to the intimal surface of the vessel.

Experimental protocol

Initially, the carotid artery was selected for our experimental model as it allows one vessel (the right carotid artery) to be used as a control and the other (the left carotid artery) to be used as a test vessel after the administration of CRL42796.

Previous studies have validated the 2-vessel model of thrombosis (Rote *et al*., 1993; 1994b). The intravenous administration of 0.9% sodium chloride solution for injection served as the placebo control, administered as a loading dose followed by a continuous infusion for 180 min. The placebo was administered 45 min before initiation of electrolytic injury in the right carotid artery. CRL42796 was administered intravenously as a loading dose of 15 μg kg^{-1} followed by an infusion of 0.31 μg kg^{-1} min^{-1} or 0.69 μg kg^{-1} min^{-1} for 180 min. The anodal current was applied to the left carotid artery 45 min after initiating the administration of CRL42796. The anodal injury current was applied for a period not to exceed 3 h. If the vessel occluded before the 3 h time point, the current was discontinued 30 min after flow in the vessel ceased. Time to occlusion was recorded. If a vessel did not occlude by 3 h, as occurred in the presence of CRL42796, the anodal current was discontinued and a value of 180 min was used for the purpose of statistical comparison.

Thrombus weight was determined after removal of the intact thrombus from the vessel wall. *Ex vivo* platelet aggregation determinations and tongue bleeding times were recorded at baseline, after completing the surgical preparation, 120 min and 225 min after placebo or drug administration. Heart rate, mean arterial blood pressure and phasic arterial blood flow were recorded continuously.

In a separate group of experiments, aspirin was administered orally at a dose of 4.6 mg kg^{-1}, 41 h and 17 h before the initiation of electrolytic injury of the vessel wall to assess the effects of combining low dose aspirin with CRL42796. Previous studies demonstrated that the aspirin dosing regimen mimics low dose aspirin and significantly increases time to arterial thrombosis (Hennan *et al*., 2001). The left carotid artery was subjected to deep vessel wall injury in the presence of low dose aspirin alone and the right carotid artery was subjected to deep vessel wall injury in the presence of a combination of low dose aspirin and CRL42796 (15 μg kg^{-1} loading dose $+0.31$ μg kg^{-1} min^{-1}).

A similar experimental protocol to that described above was used to assess the effect of CRL42796 on prevention of LCX coronary artery thrombosis except separate groups of animals acted as controls and drug-treatments. Group 1 served as controls (saline-treated i.v.), Group 2 was treated with CRL42796 (15 μg kg^{-1} loading dose $+0.31$ μg kg^{-1} min^{-1} i.v.) and Group 3 was treated with CRL42796 (15 μg kg^{-1} loading dose $+0.69$ μg kg^{-1} min^{-1} i.v.). Haematological values were obtained at baseline, 120 min and 225 min after placebo or drug administration.

Haematological measurements

Ex vivo *platelet aggregation* Blood (10 ml) was withdrawn from the right femoral vein into a plastic syringe containing 3.7% sodium citrate as the anticoagulant (1 : 10 citrate to blood; v v^{-1}). Platelet rich plasma (PRP) was obtained by collecting the supernatant from whole blood centrifuged at $140 \times g$ for 5 min. Subsequently, platelet poor plasma (PPP) was prepared from the same blood sample by further centrifugation at $2000 \times g$ for 10 min. *Ex vivo* platelet aggregation was assessed at 37°C with a four-channel platelet aggregometer (Bio-Data-PAP-4, Bio Data, Hatboro, PA, U.S.A.) by recording the increase in light transmission through a stirred suspension of PRP adjusted to 200,000 platelets μl^{-1}. Aggregation was induced with arachidonic acid (AA, 650 μM) or ADP (20 μM). A subaggregatory dose of epinephrine (550 nM) was used to prime the platelets before the agonists were added.

Tongue-template bleeding time Bleeding times were determined with the use of a Surgicutt device (International Technidyne Corporation, Edison, NJ, U.S.A.), that made a uniform incision 5 mm long and 1 mm deep on the upper surface of the tongue. The lesion was blotted with filter paper every 20 s until the transfer of blood to the filter paper ceased.

Materials

CRL42796, (2S)-2-[(2-naphthylsulphonyl)amino]-3-{[2-({4-(4-piperidinyl)-2-[2-(4-piperidinyl)ethyl]butanoyl}amino)acetyl]-amino}propanoic acid dihydrochloride was supplied by Laboratoire L. Lafon (Maisons Alfort, France) and dissolved in 0.9% sodium chloride solution for injection. The chemical structure is shown in Figure 1. All remaining reagents were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.).

Statistical analysis

The data are expressed as mean $\pm$ s.e.m. Comparisons among the incidence of carotid or coronary artery occlusion in saline and drug-treated dogs were performed using a χ^2 test. Changes in time to carotid or coronary artery thrombosis and thrombus weights between saline- and drug-treated animals were carried out using paired *t*-tests. Changes in time to thrombosis among saline-treated, aspirin-treated and aspirin plus CRL42796-treated animals were made using a one-way ANOVA followed by Student-Newman-Keuls multiple comparison test. Platelet aggregation values and bleeding times were compared to respective baseline values using a one-way ANOVA followed by Dunnet's *post hoc* analysis. $P<0.05$ was considered statistically significant for all comparisons.

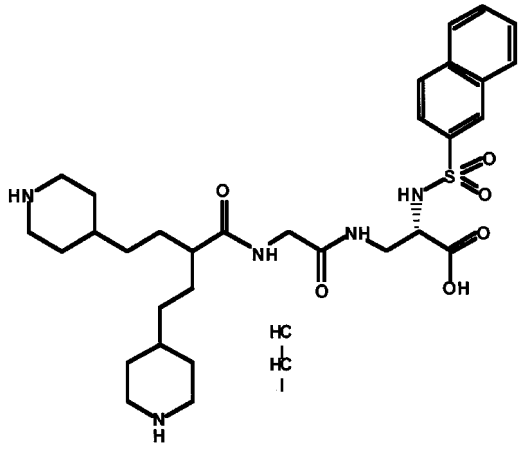

Figure 1 The chemical structure of CRL42796, (2S)-2-[(2-naphthylsulfonyl)amino]-3-{[2-({4-(4-piperidinyl)-2-[2-(4 piperidinyl)ethyl]butanoyl}amino)acetyl]amino} propanoic acid, dihydrochloride.

Results

Heart rate and mean arterial blood pressure were not different among treatment groups before administration of CRL42796. The intravenous administration of either CRL42796 or vehicle (0.9% sodium chloride solution) did not result in significant differences in heart rate and blood pressure compared to respective controls during electrolytic injury of the carotid or coronary artery (data not shown).

The effects of CRL42796 on the incidence of carotid artery thrombosis and time to occlusive thrombus development are shown in Table 1. The intravenous administration of CRL42796 (15 μg kg^{-1} loading dose +0.31 μg kg^{-1} min^{-1}) inhibited the development of occlusive thrombosis in 3/5 dogs studied and significantly increased time to thrombosis compared to saline-treated controls (CRL42796, 155.2±23.1 min; control, 47.6±8.9 min). Increasing the infusion dose of CRL42796 to 0.69 μg kg^{-1} min^{-1} completely inhibited the development of occlusive thrombosis over the 180 min of electrolytic vessel wall injury (see Table 1). Thrombus weight did not differ significantly between controls and animals receiving an infusion of CRL42796 at a rate of 0.31 μg kg^{-1} min^{-1}. However, when the infusion dose was increased to 0.69 μg kg^{-1} min^{-1} a significant decrease in thrombus weight was observed. The decrease in thrombus weight was consistent with the observation that each of the five vessels treated with the higher infusion dose of CRL42796 remained patent after 180 min of electrolytically induced vessel wall injury.

In animals pre-treated with aspirin (4.6 mg kg^{-1}) 41 and 17 hs before initiation of electrolytic injury, time to carotid artery thrombosis was increased significantly (131.0±10.7 min) compared to saline-treated controls (47.6±8.9 min) (see Table 1). Despite an increase in the time to thrombosis, aspirin did not prevent the development of an occlusive carotid artery thrombus in any of the five animals tested. When CRL42796 (15 μg kg^{-1} loading dose +0.31 μg kg^{-1} min^{-1} i.v.) was combined with low dose aspirin, three of the five vessels tested did not occlude during 180 min of electrolytic injury. It is notable that similar results were observed in the absence of low dose aspirin (see Table 1). Therefore, it appears that combining low dose aspirin with CRL42796 does not provide additional protection against the development of occlusive arterial thrombosis. Low dose aspirin or a combination of low dose aspirin and CRL42796 did not produce a significant change in thrombus weight compared to respective controls.

Figure 2 illustrates the carotid artery blood flow in control or CRL42796-treated animals during induction of vessel wall injury. Time zero on the graph represents the initiation of the anodal current for induction of deep vessel wall injury. As indicated in Figure 2a, control animals exhibited a progressive decline in carotid artery blood flow to zero. The latter was accompanied by the development of an occlusive thrombus. In the presence of CRL42796 (15 μg kg^{-1} loading dose +0.31 μg kg^{-1} min^{-1} i.v.) a progressive decrease in blood flow was observed during electrolytic injury, however three of the five carotid arteries remained patent throughout the 180 min duration of anodal current application. When the intravenous infusion of CRL42796 was increased to 0.69 μg kg^{-1} min^{-1} the carotid artery blood flow remained relatively stable during application of the electrolytic injury current and all vessels were patent at the end of 180 min at which point the anodal current was terminated (see Figure 2b).

When aspirin (4.6 mg kg^{-1} −41, −17 h p.o.) was combined with CRL42796 (15 μg kg^{-1} loading dose +0.31 μg kg^{-1} min^{-1} i.v.) carotid artery blood flow decreased progressively during electrolytic injury. However, despite the addition of aspirin to the treatment regimen only three of the five vessels studied remained patent over the course of the experiment (see Figure 2c).

The effect of CRL42796 on platelet aggregation in response to ADP (20 μM) and arachidonic acid (AA, 650 μM) from citrated whole blood is shown in Figure 3. At doses of 15 μg kg^{-1} loading dose +0.31 μg kg^{-1} min^{-1} i.v. (Figure 3a) and 15 μg kg^{-1} loading dose +0.69 μg kg^{-1} min^{-1} i.v. (Figure 3b), CRL42796 significantly reduced *ex vivo* platelet responses to both ADP and AA. When animals were pretreated with aspirin (4.6 mg kg^{-1} −41, 17 h p.o.), platelet responses to AA were inhibited at baseline indicating inhibition of platelet COX-1. In contrast, platelet responses to ADP were unchanged (see Figure 3c). As anticipated, the addition of CRL42796 (15 μg kg^{-1} loading dose +0.31 μg kg^{-1} min^{-1} i.v.) significantly reduced, but did not prevent, *ex vivo* platelet responses to ADP in aspirin-treated dogs, whereas *ex vivo* platelet responses to AA remained inhibited over the course of the experimental protocol.

Selected experiments were conducted to determine if platelet responses are altered by the use of citrate as an

Table 1 Incidence of carotid artery occlusion and time to thrombosis after intravenous administration of CRL42796

Treatment	*Carotid artery occlusion (incidence) (%)*	*Carotid artery time to thrombosis* (min)	*Thrombus weight* (mg)
Saline (loading dose + infusion i.v.)	5/5 (100)	47.6±8.9	20.2±4.1
CRL42796 (15 μg kg^{-1} loading dose + 0.31 μg kg^{-1} min^{-1} i.v.)	2/5 (40)	155.2±23.1*	27.6±6.6
Saline (loading dose + infusion i.v.)	5/5 (100)	47.2±6.5	27.5±1.4
CRL42796 (15 μg kg^{-1} loading dose + 0.69 μg kg^{-1} min^{-1} i.v.)	0/5 (0)*	180.0±0*	6.1±1.7*
Aspirin (4.6 mg kg^{-1} −41, −17 h p.o.) Saline (loading dose + infusion i.v.)	5/5 (100)	131.0±10.7*	22.2±5.2
Aspirin (4.6 mg kg^{-1} −41, −17 h p.o.) CRL42796 (15 μg kg^{-1} loading dose + 0.31 μg kg^{-1} min^{-1} i.v.)	2/5 (40)	154.6±16.2*	18.8±3.3

Data represent mean±s.e.mean for $n=5$ experiments. *Indicates a significant difference from respective saline control ($P<0.05$).

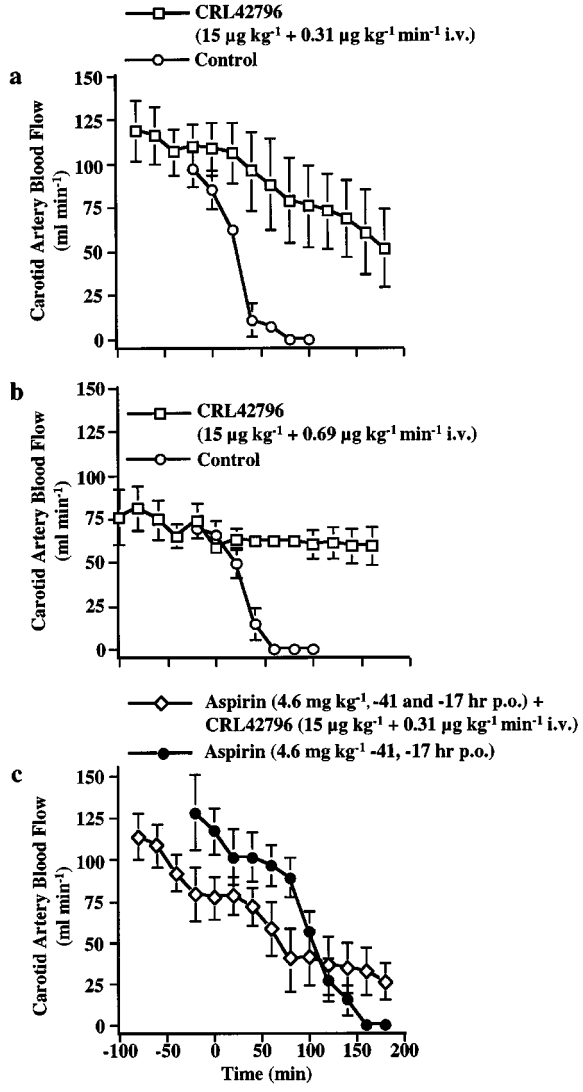

Figure 2 Carotid artery blood flow during electrolytic injury in the absence and presence of CRL42796 (15 μg kg^{-1} + 0.31 μg kg^{-1} min^{-1} i.v.) (a); (15 μg kg^{-1} + 0.69 μg kg^{-1} min^{-1} i.v.) (b); and a combination of aspirin (4.6 mg kg^{-1} −41, −17 h p.o. + 15 μg kg^{-1} + 0.31 μg kg^{-1} min^{-1} i.v.) (c). Deep vessel wall injury was initiated at time 0 and produced complete and persistent occlusion of all control carotid arteries. CRL42796 maintained carotid artery patency in all vessels for the duration of the infusion at a dose of 0.69 μg kg^{-1} min^{-1} i.v. and in three of five vessels at a dose of 0.31 μg kg^{-1} min^{-1} i.v. The combination of aspirin and the lower infusion dose of CRL42796 produced no additional benefit. Values are mean ± s.e.m for $n = 5$ experiments.

anticoagulant. Platelet responses to ADP and AA were assessed under conditions in which venous blood for the preparation of PRP and PPP was collected in syringes in which heparin served as the anticoagulant. Figure 4a illustrates the effects of CRL42796 (15 μg kg^{-1} loading dose + 0.31 μg kg^{-1} min^{-1} i.v.) on platelet aggregation in response to AA and ADP when heparin was the anticoagulant. The platelet aggregation responses were reduced in the presence of the lower dose of CRL42796, however the changes were not statistically significant. At the higher dose of CRL42796, a significant reduction in *ex vivo* platelet reactivity was observed. In aspirin-treated animals (4.6 mg kg^{-1} −41, −17 h p.o.), the use of heparin as an anticoagulant produced similar results as observed with citrate. AA-induced platelet aggregation was inhibited significantly at baseline compared to non-aspirin treated controls and remained inhibited throughout the experimental protocol. ADP-induced platelet aggregation in aspirin-treated dogs was similar at baseline with heparin as the anticoagulant and the addition of CRL42796 significantly reduced platelet reactivity (see Figure 4c).

The effects of CRL42796 on tongue bleeding time are shown in Figure 5. At the lower infusion dose of CRL42796 there was a trend towards an increase in tongue bleeding time; however, the change was not statistically significant from baseline (Figure 5a). When the infusion dose of CRL42796 was increased to 0.69 μg kg^{-1} min^{-1}, there was a related significant increase in tongue bleeding time at 120 and 240 min after initiating the drug infusion (see Figure 5b). Bleeding times exceeding 10 min were recorded as 10 min for statistical analysis.

Aspirin (4.6 mg kg^{-1} −41, −17 h p.o.) did not produce a significant increase in bleeding time at baseline compared to non-treated control animals (Figure 5c). The combination of aspirin and CRL42796 (15 μg kg^{-1} loading dose + 0.31 μg kg^{-1} min^{-1} i.v.) did not induce an increase in bleeding time.

The effects of CRL42796 on the incidence of left circumflex coronary artery thrombosis and time to occlusive thrombus development are shown in Table 2. At an intravenous dose of 15 μg kg^{-1} loading dose + 0.31 μg kg^{-1} min^{-1} infusion, CRL42796 inhibited the development of occlusive thrombosis in 4/5 dogs and significantly increased time to thrombosis compared to saline-treated controls (CRL42796, 165.8 ± 14.2 min; control, 90.8 ± 10.4 min). Increasing the infusion dose to 0.69 μg kg^{-1} min^{-1} completely inhibited the development of occlusive thrombosis over the 180 min period of electrolytic vessel wall injury (see Table 2). Thrombus weight was not significantly different among control and CRL42796-treated animals.

The *in vivo* antithrombotic effects of CRL42796 were examined in the canine coronary artery subjected to deep vessel wall injury. The blood flow results are summarized in Figure 6. In the control group ($n = 5$), coronary artery blood flow decreased progressively commencing with the induction of electrolytic injury of the vessel wall. Occlusive thrombosis developed in each of the vessels within 90.8 ± 10.4 min. Two separate groups of five animals each received CRL42796 commencing before induction of vessel wall injury. As before, the drug was administered as an intravenous loading dose of 15 μg kg^{-1} followed by an infusion of either 0.31 μg kg^{-1} min^{-1} ($n = 5$) or 0.69 μg kg^{-1} min^{-1} ($n = 5$). As indicated in Table 2, one of five vessels occluded in the group receiving the low infusion dose whereas all vessels remained patent in the group given the high infusion dose. Although all vessels remained patent in the high infusion dose group, the time to occlusion is listed in Table 2 as 180 ± 0 min for the purpose of statistical analysis.

Occlusive and non-occlusive thrombi were found in the injured coronary arteries. In the saline-treated control group, occlusive thrombi were present in each of the five arteries and firmly attached to the site of vessel injury. One of five vessels

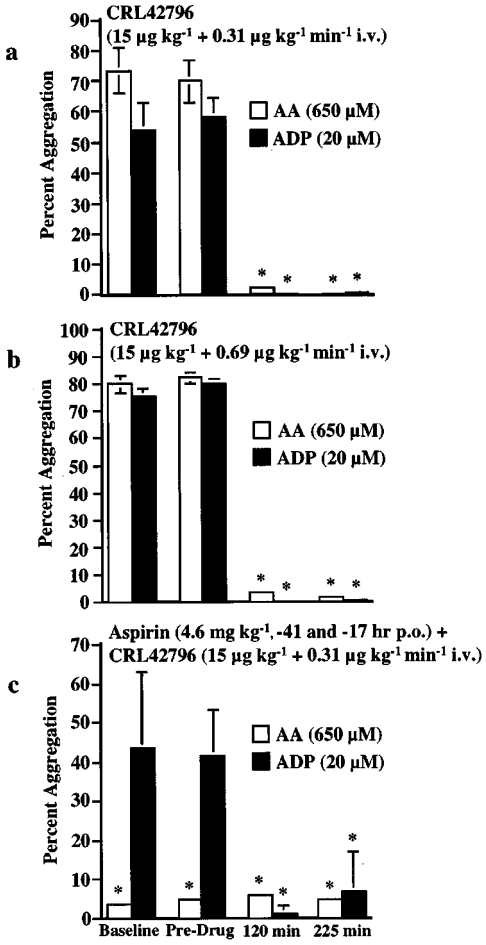

Figure 3 Effect of intravenous administration of CRL42796 on citrated *ex-vivo* platelet aggregation induced by AA (650 μM) and ADP (20 μM) during electrolytic injury to the carotid artery. CRL42796 was administered as a loading dose of 15 μg kg^{-1} followed by either 0.31 μg kg^{-1} min^{-1} (a) or 0.69 μg kg^{-1} min^{-1} (b). In (c), the effect of combining aspirin (4.6 mg kg^{-1} -41, -17 h p.o.) with the lower infusion dose of CRL42796 are shown. Values are expressed as mean$\pm$s.e.m for $n=5$ experiments. Asterisks indicate a significant reduction in platelet responses compared to respective baseline ($P<0.05$).

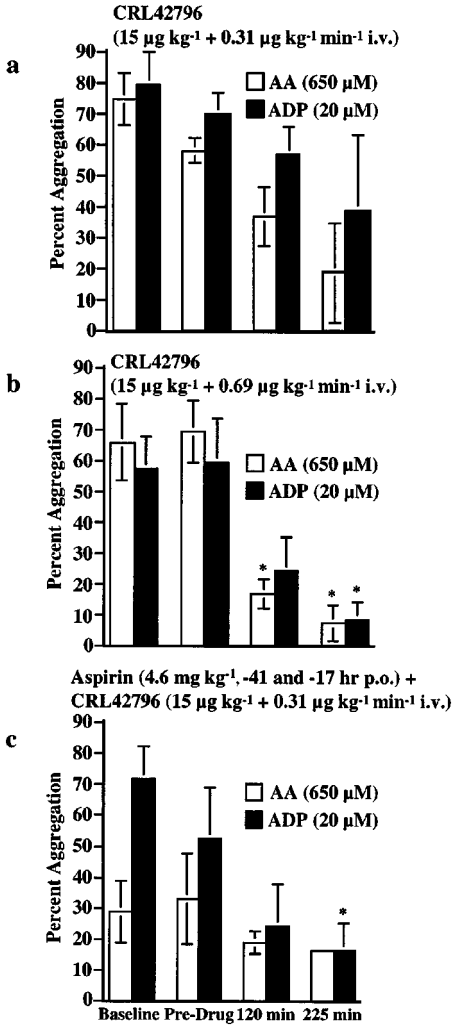

Figure 4 Effect of intravenous administration of CRL42796 on heparinized *ex-vivo* platelet aggregation induced by AA (650 μM) and ADP (20 μM) during electrolytic injury to the carotid artery. CRL42796 was administered as a loading dose of 15 μg kg^{-1} followed by either 0.31 μg kg^{-1} min^{-1} (a) or 0.69 μg kg^{-1} min^{-1} (b). In (c), the effect of combining aspirin (4.6 mg kg^{-1} -41, -17 h p.o.) with the lower infusion dose of CRL42796 are shown. Values are expressed as mean$\pm$s.e.m for $n=5$ experiments. Asterisks indicate a significant reduction in platelet responses compared to respective baseline ($P<0.05$).

in the low dose infusion group showed the presence of an occlusive thrombus while occlusive thrombi were not found in any of the vessels in the high dose infusion group. The thrombus weights did not differ among the groups despite the fact that control vessels occluded whereas nine of ten vessels from the animals treated with CRL42796 remained patent. The apparent discrepancy between thrombus weight and vessel patency relates to the fact that flow was preserved in the presence of the antiplatelet agent. Inhibition of the platelet GPIIb/IIIa receptor prevents the formation of an occlusive platelet plug and results in a thrombus mass that extends in a retrograde direction without occluding the vessel lumen. Prolongation in the time to thrombosis or its prevention provides an extended period over which the 'tail' of the thrombus can propagate before the experiment is terminated. In the control group, platelet accretion results in the rapid formation of a firm, occlusive platelet plug with little opportunity for extension beyond the site of vessel wall injury before flow declines to zero at which point the vessel is removed for determination of thrombus weight.

Ex vivo platelet responses in citrated whole blood from the coronary artery thrombosis protocol are shown in Figure 7. PRP prepared from blood of control (saline-treated) dogs showed typical *ex vivo* aggregation responses to AA (Figure 7a) or ADP (Figure 7b) over the entire time course of the experimental protocol. Treatment with CRL42796 significantly reduced platelet responses to both AA and ADP at 120 and 225 min after administration CRL42796 in both the high and low dose regimens (see Figure 7). *Ex vivo* platelet aggregation determinations also were performed with heparin as the anticoagulant. As shown in Figure 8, *ex vivo* platelet responses to AA (Figure 8a) and ADP (Figure 8b) were reduced after administration of CRL42796 compared to

baseline; however not all measures were significantly different from baseline as was observed with citrated PRP.

The effects of CRL42796 on bleeding time during the coronary artery thrombosis experiments are shown in Figure 9. After a loading dose of CRL42796 (15 μg kg^{-1}), infusion doses of 0.31 μg kg^{-1} min^{-1} and 0.69 μg kg^{-1} min^{-1} increased tongue bleeding time significantly compared to non-treated controls. The increase in bleeding time was similar at 120 min and 225 min after initiation of the infusion.

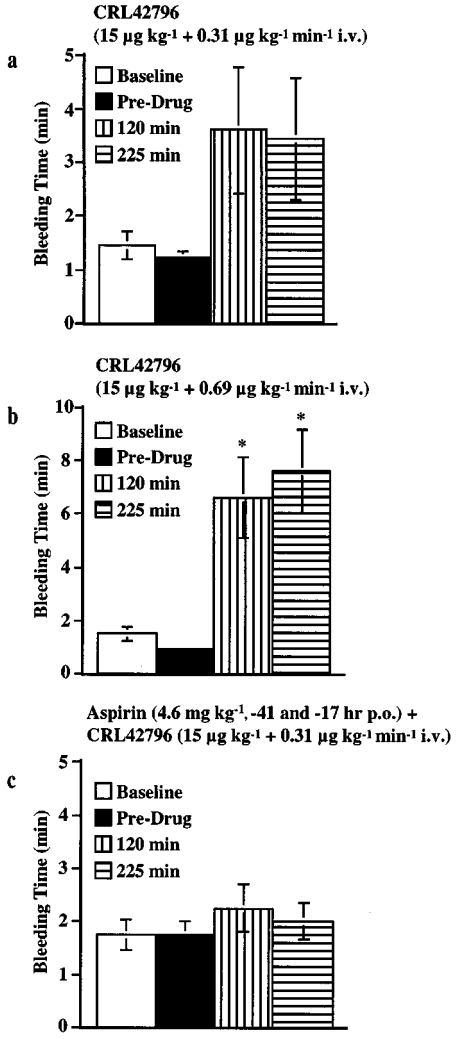

Figure 5 Effect of CRL42796 on tongue bleeding time at baseline, after surgery, and 120 min and 225 min after intravenous administration of drug. CRL42796 was administered as a loading dose of 15 μg kg^{-1} followed by either 0.31 μg kg^{-1} min^{-1} (a) or 0.69 μg kg^{-1} min^{-1} (b). In (c), the effect of combining aspirin (4.6 mg kg^{-1} -41, -17 h p.o.) with the lower infusion dose of CRL42796 are shown. Values are expressed as mean $\pm$ s.e.m for $n=5$ experiments. Asterisks indicate a significant increase in bleeding time compared to respective baseline ($P<0.05$).

Discussion

The experimental model of arterial thrombosis used in this study was validated previously (Romson *et al*., 1980; Rote *et al*., 1993; 1994b). The unique feature of the model is that arterial thrombosis develops spontaneously in response to deep vessel wall injury. The latter is the dominant influence and by itself can lead to thrombosis. The vascular injury is induced by an anodal current applied to the endothelial surface of the vessel resulting in exposure of subendothelial collagen and other components capable of increasing platelet reactivity and subsequent activation of the coagulation cascade. The development of a deep arterial wall lesion and exposure of subendothelial structures allows the previously nonthrombotic vascular surface to become prothrombotic. The imposition of a critical stenosis immediately distal to the site of vessel wall injury contributes to the thrombotic process by increasing the degree of turbulence. The disruption in the normal pattern of blood flow further impairs endothelial function and forms countercurrents and local pockets of stasis. Turbulence and stasis disrupt the normal pattern of laminar flow thereby allowing platelets to encounter the prothrombotic surface of the injured vessel wall. Within a brief period after endothelial injury, platelets begin to form a monolayer at the site where subendothelial elements encounter the flowing blood. Subsequently the progressive accretion of platelets, formation of fibrin and entrapment of cellular blood components leads to the development of a platelet-rich thrombus supported by a firm fibrin network (Romson *et al*., 1980; Bush & Shebuski, 1990). A detailed electron-microscopic examination of the electrolytically-induced vessel wall injury and cellular composition of the thrombus, plus the benefits derived from inhibition of *in vivo* platelet reactivity has been published (Romson *et al*., 1980).

Many experimental models have been used for the study of antithrombotic drugs (Bush & Shebuski, 1990). In the electrolytic injury model, development of an occlusive thrombus proceeds spontaneously in response to vessel wall injury. The ability to control the size of the injured area, plus the intensity and duration of the electrolytic anodal current, make the model highly reproducible and dependent upon natural physiological processes that lead to thrombus formation. The interaction between the injured vessel wall

Table 2 Incidence of left circumflex coronary artery occlusion and time to thrombosis after treatment with CRL42796

Treatment	*Coronary artery occlusion (incidence) (%)*	*Carotid artery time to thrombosis* (min)	*Thrombus weight* (mg)
Control (saline + infusion i.v.)	5/5 (100)	90.8 $\pm$ 10.4	8.8 $\pm$ 2.3
CRL42796 (15 μg kg^{-1} + 0.31 μg kg^{-1} min^{-1} i.v.)	1/5 (20)*	165.8 $\pm$ 14.2*	7.9 $\pm$ 2.6
CRL42796 (15 μg^{-1} kg + 0.69 μg kg^{-1} min^{-1} i.v.)	0/5 (0)*	180.0 $\pm$ 0*	12.4 $\pm$ 0.9

Data represent mean $\pm$ s.e.mean for $n=5$ or 6 experiments. *Indicates a significant difference from saline-treated control ($P<0.05$).

and the circulating blood components are similar to those pathophysiologic mechanisms that participate in the formation of occlusive thrombi in humans with vascular disease. The ability to better approximate the pathophysiologic events

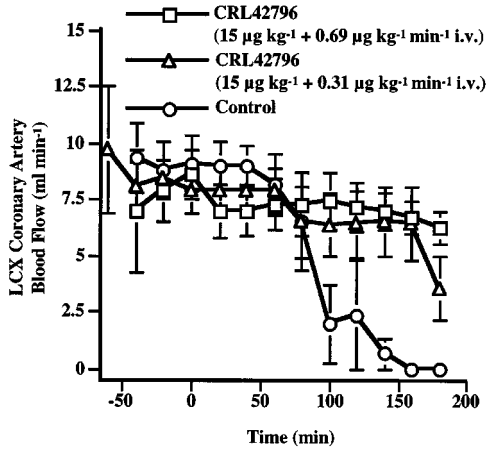

Figure 6 Left circumflex coronary artery blood flow during electrolytic injury. Deep vessel wall injury resulted in thrombotic occlusion of all control vessels. Administration of CRL42796 maintained vessel patency in all vessels treated with a 15 μg kg^{-1} loading dose followed by a 0.69 μg kg^{-1} min^{-1} i.v. infusion and in four of five vessels treated with the lower infusion dose of 0.31 μg kg^{-1} min^{-1} i.v. Values are expressed as mean$\pm$s.e.m for $n=5$ or six experiments.

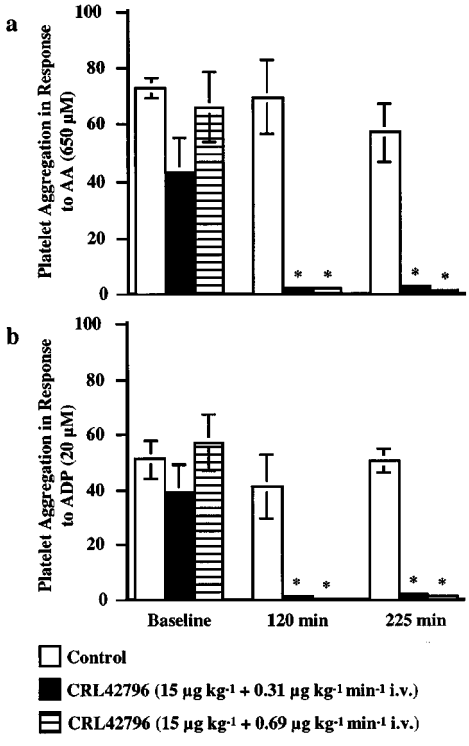

Figure 7 Effect of intravenous administration of CRL42796 on citrated platelet responses to AA (650 μM) (a) and ADP (20 μM) (b) during electrolytic injury to the left circumflex coronary artery. CRL42796 was administered as a loading dose of 15 μg kg^{-1} followed by either 0.31 μg kg^{-1} min^{-1} (a) or 0.69 μg kg^{-1} min^{-1} (b). Values are expressed as mean$\pm$s.e.m for $n=5$ or six experiments. Asterisks indicate a significant reduction in platelet responses compared to respective baseline ($P<0.05$).

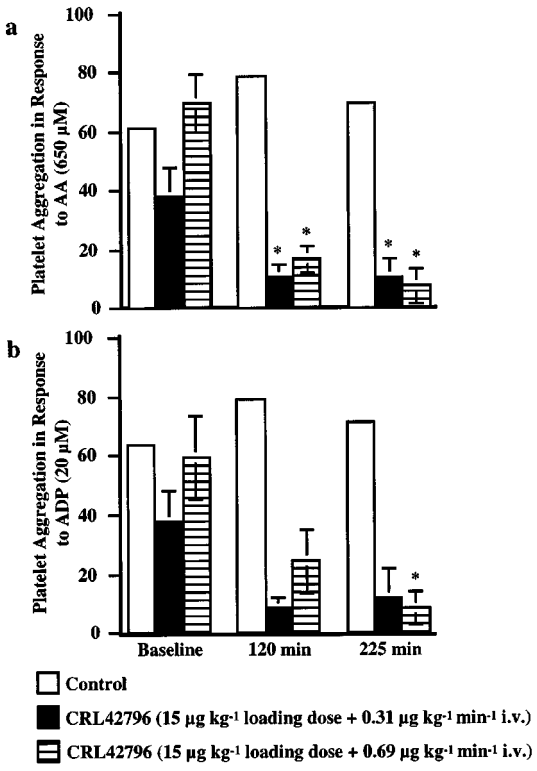

Figure 8 Effect of intravenous administration of CRL42796 on heparinized platelet responses to AA (650 μM) (a) and ADP (20 μM) (b) during electrolytic injury to the left circumflex coronary artery. CRL42796 was administered as a loading dose of 15 μg kg^{-1} followed by either 0.31 μg kg^{-1} min^{-1} (a) or 0.69 μg kg^{-1} min^{-1} (b). Values are expressed as mean$\pm$s.e.m for $n=5$ or six experiments. Asterisks indicate a significant reduction in platelet responses compared to respective baseline ($P<0.05$).

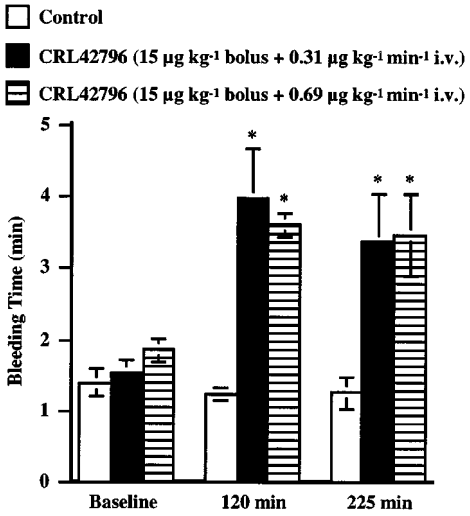

Figure 9 Effect of CRL42796 on tongue bleeding time at baseline and 120 min and 225 min after intravenous administration of drug during electrolytic injury to the left circumflex coronary artery. Values are expressed as mean$\pm$s.e.m for $n=5$ or six experiments. Asterisks indicate a significant increase in bleeding time compared to respective baseline ($P<0.05$).

known to occur clinically provides a reliable model for the evaluation of antiplatelet agents for the prevention of occlusive arterial thrombosis.

During the complex platelet response to vessel wall injury, multiple mediators contribute to platelet activation and aggregation including fibrinogen, thrombin, ADP, thromboxane A2 and serotonin (Marcus, 1994). Conventional antithrombotic agents such as aspirin, prostacyclin, thromboxane synthase inhibitors and thromboxane receptor antagonists directly target single mediators of platelet activation. Because the final common pathway in thrombus development involves platelets binding to fibrinogen *via* the GPIIb/IIIa receptor, inhibition of this final pathway provides an effective antithrombotic effect that encompasses inhibition of platelet activation in response to numerous platelet agonists. The present study demonstrates the *in vivo* efficacy of the platelet GPIIb/IIIa receptor antagonist CRL42796 in an electrolytic injury model of the canine carotid and coronary artery.

The intravenous threshold dose of CRL42796 for preventing arterial thrombosis required a loading dose of 15 μg kg^{-1} followed by a continuous infusion of 0.31 μg kg^{-1} min^{-1} or 0.69 μg kg^{-1} min^{-1}. Both infusion rates maintained vessel patency with the higher rate being more effective. There was a progressive decline in carotid artery blood flow over the course of the experiment with the lower infusion dose that was not observed when the dose was increased. Thrombi recovered from the vessels subjected to electrolytic injury during treatment with CRL42796 were smaller than those retrieved from control vessels which correlates well with the inhibition of occlusive thrombus formation. However, these results should be interpreted carefully as the thrombi in control arteries were *in situ* for a longer period and had an opportunity to increase in size. It is important to recall that arterial thrombi propagate in a retrograde direction due to the presence of an occlusive plug and stasis. The events leading to thrombus propagation would be eliminated or markedly reduced under conditions in which blood flow is maintained, albeit reduced, after treatment with an antiplatelet agent.

Ex vivo platelet responses from citrate anticoagulated whole blood were only partly correlated with the inhibition of arterial thrombosis. The high and low infusion rate of CRL42796 completely inhibited platelet responses as long as the infusion was maintained; however, two of the five vessels tested at the lower infusion dose developed occlusive thrombosis. Previous studies have indicated that citrated whole blood platelet responses after treatment with GPIIb/IIIa receptor antagonists do not correlate with the *in vivo* prevention of arterial thrombosis (Phillips *et al*., 1997; Rebello *et al*., 1998). The discrepancy between the *in vitro* platelet aggregation responses conducted in the presence of citrate and the *in vivo* results relates to a decrease in the plasma ionized calcium concentration due to chelation with citrate (Phillips *et al*., 1997; Rebello *et al*., 1998). Calcium is essential for the formation of the GPIIb/IIIa heterodimer complex (Lam, 1992) and for the interaction between the GPIIb/IIIa receptor and fibrinogen (Steiner *et al*., 1989). When the calcium concentration of platelet rich plasma is decreased by citrate, binding of the GPIIb/IIIa receptor antagonist is enhanced resulting in an overestimation of the antiplatelet efficacy of the compound. In animals treated with the lower infusion dose of CRL42796 (0.31 μg kg^{-1} min^{-1}) platelet responses in PRP prepared from heparin anticoagulated whole blood were reduced, but not significantly inhibited. With the lower infusion dose of CRL42796 40% of the vessels occluded. Thus, it appears that *ex vivo* platelet aggregation responses conducted in heparin anticoagulated PRP are better correlated more dependably with the *in vivo* observations on thrombosis. At the higher infusion dose of CRL42796 (0.69 μg kg^{-1} min^{-1}) platelet responses from heparin anticoagulated PRP were significantly reduced and thrombus formation was prevented. Thus, effective antithrombotic doses of GPIIb/IIIa receptor antagonists may be extrapolated more accurately from *ex vivo* platelet aggregation responses performed in PRP in which the physiologic concentration of ionized calcium is maintained.

An interesting aspect of the platelet inhibitory action of CRL42796 was that it dissipated rapidly upon discontinuing the drug infusion and vessels occluded within 60 min of terminating the infusion (data not shown). Thus, CRL42796 displays a rapid-on and rapid-off pharmacological profile that may offer an improved safety factor in those clinical situations where increased bleeding might occur. Other GPIIb/IIIa receptor antagonists such as 7E3 (Bates *et al*., 1992; Rote *et al*., 1994c), DMP-728 (Lucchesi *et al*., 1994), and SM-20302 (Huang *et al*., 1999) result in extended maintenance of vessel patency despite the fact that *ex vivo* platelet aggregation responses return to baseline values. There are other factors to consider involving a time-dependent decrease in the thrombogenicity of the injured vessel wall thus allowing preservation of blood flow in the presence of normal platelet function. The pharmacokinetic profile of CRL42796 may be of value when used over a period of 24–48 h to provide sufficient time for vessel wall 'passivation' to occur before terminating the infusion (Bates *et al*., 1992).

Bleeding times have been reported to be a poorly reproducible determinant of platelet reactivity (Channing-Rodgers & Levine, 1990). In the present study, tongue-bleeding times correlated with the inhibition of platelet activity and thrombosis. At the lower infusion dose of CRL42796, we did not observe a significant increase in bleeding and occlusive thrombus formation was not prevented completely. At the higher infusion dose, tongue-bleeding time was increased significantly and occlusive thrombosis was inhibited. The results suggest that bleeding time may be a useful measure for the ability of a platelet receptor antagonist to inhibit arterial thrombosis.

The combination of CRL42796 and aspirin failed to produce an additive or synergistic antithrombotic effect. Aspirin was administered orally at -41 and -17 h before commencing the experimental protocol. Aspirin is known to irreversibly inhibit platelet derived COX-1 and to reversibly inhibit the constitutive and inducible forms of endothelial COX-2 (Vane, 1971).

We previously demonstrated that the dosing regimen for aspirin as used in this study inhibits platelet responses to arachidonic acid while allowing endothelial sources of COX-2 to regenerate and produce prostacyclin (Hennan *et al*., 2001). Aspirin alone significantly increased time to thrombosis in the current study; however, it did not prevent occlusive arterial thrombus formation. This result is consistent with those observed previously in this laboratory using the same experimental model and aspirin-dosing regimen (Hennan *et*

al., 2001). When aspirin was combined with the low infusion dose of CRL42796, no additional antithrombotic benefit was observed. Time to thrombosis remained relatively the same and two of the five vessels subjected to electrolytic injury occluded. CRL42796 administered at the same dose in the absence of aspirin resulted in occlusion in two of five vessels. An analysis of the carotid artery blood flow in animals treated with aspirin and CRL42796 revealed a rapid decline in flow over the 180 min of electrolytic injury. In comparison to treatment with CRL42796 alone, the addition of aspirin appeared to accelerate the reduction in blood flow that occurs in response to deep vessel wall injury. The underlying mechanism to account for this observation remains unknown and is deserving of further study. It is interesting to note that bleeding times were unchanged in the presence of aspirin and CRL42796, possibly indicating a change in vascular tone in the tongue. On the other hand, one might question the benefit to be derived from the addition of aspirin to a treatment regimen involving the use of a platelet GPIIb/IIIa receptor antagonist. The antiplatelet effect of aspirin is directed against the enzymatic conversion of arachidonic acid to thromboxane A_2 in which the latter serves as an agonist for platelet aggregation. Aspirin does not prevent platelet aggregation by other agonists present *in vivo* during vessel wall injury. Furthermore, since activation of the GPIIb/IIIa receptor is the final common pathway for platelet aggregation, it is unlikely that aspirin would serve as an effective adjunctive agent.

In conclusion, CRL42796 effectively inhibits *ex vivo* platelet aggregation in response to AA and ADP and only marginally increases bleeding time. CRL42796 is an effective *in vivo* intravenous antithrombotic agent for preventing occlusive arterial thrombosis in response to deep vessel wall injury when used as the sole intervention. *Ex vivo* platelet aggregation responses conducted in the presence of a physiologic plasma ionized calcium concentration is a more accurate indicator of a drug's antithrombotic potential compared to *ex vivo* studies done in PRP anticoagulated with citrate. As the dose of CRL42796 is increased bleeding time increases; however, careful control of the plasma concentration may help to avoid excessive peak plasma levels that contribute to excessive bleeding. The combination of low dose CRL42796 with aspirin did not provide a further benefit. Furthermore, the addition of aspirin appears to paradoxically reduce the efficacy of CRL42796. The mechanism to account for this finding is not apparent and is deserving of further investigation.

This study was supported by a grant from Laboratoire L. Lafon and the Cardiovascular Pharmacology Fund, University of Michigan. J.K. Hennan was supported by a postdoctoral research fellowship from the Heart and Stroke Foundation of Canada. CRL42796 was provided by Laboratoire L. Lafon, Centre de Recherches (Maisons Alfort, France).

References

BATES, E.R., WALSH, D.G., MU, D.-X., ABRAMS, G.D. & LUCCHESI, B.R. (1992). Sustained inhibition of vessel wall-platelet interaction after deep coronary artery injury by temporary inhibition of the platelet glycoprotein IIb/IIIa receptor. *Coronary Art. Dis.,* **3,** 67 – 76.

BUSH, L.R. & SHEBUSKI, R.J. (1990). In vivo models of arterial thrombosis and thrombolysis. *FASEB J.,* **4,** 3087 – 3098.

CHANNING-RODGERS, R.P. & LEVINE, J. (1990). A critical reappraisal of the bleeding time. *Semin. Thromb. Hemost.,* **16,** 1 – 20.

GENSINI, G.F., COMEGLIO, M. & FALAI, M. (1999). Advances in antithrombotic therapy of acute myocardial infarction. *Am. Heart J.,* **138,** 171 – 176.

GIBSON, C.M. (1999). Primary angioplasty compared with thrombolysis: new issues in the era of glycoprotein IIb/IIIa inhibition and intracoronary stenting. *Ann. Intern. Med.,* **130,** 841 – 847.

HENNAN, J.K., HUANG, J., BARRETT, T.D., DRISCOLL, E.M., WILLENS, D.E., PARK, A.M., CROFFORD, L.J. & LUCCHESI, B.R. (2001). Effects of selective cyclooxygenase-2 inhibition on vascular responses and thrombosis in canine coronary arteries. *Circulation,* **104,** 820 – 825.

HUANG, J., REBELLO, S.S., ROSENBURG, L.A., KANEKO, M., SAKURAMA, T. & LUCCHESI, B.R. (1999). Temporary and partial inhibition of platelets by SM-20302 prevents coronary artery thrombosis in a chronic canine model. *Eur. J. Pharmacol.,* **366,** 203 – 213.

LAM, S.C. (1992). Isolation and characterization of a chymotryptic fragment of platelet glycoprotein IIb/IIIa retaining Arg-Gly-Asp binding activity. *J. Biol. Chem.,* **267,** 5649 – 5655.

LUCCHESI, B.R., ROTE, W.E., DRISCOLL, E.M. & MU, D.-X. (1994). Prevention of thrombosis and rethrombosis and enhancement of the thrombotic actions of recombinant tissue-type plasminogen activator in the canine heart by DMP728, a glycoprotein IIb/IIIa antagonist. *Br. J. Pharmacol.,* **113,** 1333 – 1343.

MAKKAR, R.R., LITVACK, F., EIGLER, N.L., NAKAMURA, M., IVEY, P.A., FORRESTER, J.S., SHAH, P.K., JORDAN, R.E. & KAUL, S. (1997). Effects of GPIIb/IIIa receptor monoclonal antibody (7E3), heparin, and aspirin in an ex vivo canine arteriovenous shunt model of stent thrombosis. *Circulation,* **95,** 1015 – 1021.

MARCUS, A.J. (1994). Cellular interactions of platelets in thrombosis. In *Thrombosis and hemorrhage*. ed. Loscalzo, J. & Schafer, A.I. pp 279 – 291. Boston: Blackwell.

MICKELSON, J.K., SIMPSON, P.J., CRONIN, M., HOMEISTER, J.W., LAYWELL, E., KITZEN, J. & LUCCHESI, B.R. (1990). Antiplatelet antibody [7E3 F(ab′)2] prevents rethrombosis after recombinant tissue-type plasminogen activator-induced coronary artery thrombolysis in a canine model. *Circulation,* **81,** 617 – 627.

PHILLIPS, D.R., TENG, W., ARFSTEN, A., NANNIZZI-ALAIMO, L., WHITE, M.M., LONGHURST, C., SHATTIL, S.J., RANDOLPH, A., JAKUBOWSKI, J.A., JENNINGS, L.K. & SCARBOROUGH, R.M. (1997). Effect of calcium on GPIIb/IIIa interactions with integrilin: enhanced GPIIb/IIIa binding and inhibition of platelet aggregation by reductions in the concentration of ionized calcium in plasma anticoagulated with citrate. *Circulation,* **96,** 1488 – 1494.

REBELLO, S.S., HUANG, J., SAITO, K. & LUCCHESI, B.R. (1998). The *in vivo* efficacy of SM-20302, a GPIIb/IIIa receptor antagonist, correlates with ex vivo platelet inhibition in heparinized blood but not in citrated blood. *Arterioscler. Thromb. Vasc. Biol.,* **18,** 954 – 960.

ROMSON, J.L., HAACK, D.W. & LUCCHESI, B.R. (1980). Electrical induction of coronary artery thrombosis in the ambulatory canine: a model for in vivo evaluation of antithrombotic agents. *Thromb. Res.,* **17,** 841 – 853.

ROTE, W.E., DAVIS, J.H., MOUSA, S.A., REILLY, R.M. & LUCCHESI, B.R. (1994b). Antithrombotic effects of DMP-728, a platelet GPIIb/IIIa receptor antagonist, in a canine model of arterial thrombosis. *J. Cardiovasc. Pharmacol.,* **23,** 681 – 689.

ROTE, W.E., MU, D-X., BATES, E.R., NEDLEMAN, M.A. & LUCCHESI, B.R. (1994c). Prevention of rethrombosis after coronary thrombolysis in a chronic canine model: I. Adjunctive therapy with monoclonal antibody 7E3 F(ab′)2 fragment. *J. Cardiovasc. Pharmacol.,* **23,** 194 – 202.

ROTE, W.E., NEDELMAN, M.A., MU, D.-X., MANLEY, P.J., WEISMAN, H., CUNNINGHAM, M.R. & LUCCHESI, B.R. (1994a). Chimeric 7E3 prevents carotid artery thrombosis in cynomolgus monkeys. *Stroke,* **25,** 1223 – 1232.

ROTE, W.E., WERNS, S.E., DAVIS, J.H., FEIGEN, L.P., KILGORE, K.S. & LUCCHESI, B.R. (1993). Platelet GPIIb/IIIa receptor inhibition by SC-49992 prevents thrombosis and rethrombosis in the canine carotid artery. *Cardiovasc. Res.,* **27,** 500 – 507.

SPINLER, S.A., HILLEMAN, D.E., CHENG, J.W., HOWARD, P.A., MAURO, V.F., LOPEZ, L.M., MUNGER, M.A., GARDNER, S.F. & NAPPI, J.M. (2001). New recommendations from the 1999 American College of Cardiology/American Heart Association acute myocardial infarction guidelines. *Ann. Pharmacother.,* **35,** 589 – 617.

STEINER, B., COUSAT, D., TRZECIAK, A., GILLESSEN, D. & HADVARY, P. (1989). Calcium-dependent binding of a synthetic Arg-Gly-Asp (RGD) peptide to a single site on the purified platelet glycoprotein IIb/IIIa complex. *J. Biol. Chem.,* **264,** 13102 – 13108.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature New Biol.,* **231,** 232 – 235.

(Received January 4, 2002
Revised March 20, 2002
Accepted March 27, 2002)