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Research Article

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Coronary Vascular Occlusion Mediated Via Thromboxane A₂-Prostaglandin Endoperoxide Receptor Activation In Vivo

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Abstract

The use of enzyme inhibitors to clarify the role of thromboxane A₂ in vasoocclusive disease has been complicated by their non-specific action. To address this problem we have examined the effects of thromboxane A₂/prostaglandin endoperoxide receptor antagonism in a canine model of platelet-dependent coronary occlusion. Two structurally distinct thromboxane A₂/prostaglandin endoperoxide receptor antagonists, 3-carboxyl-dibenzo (b, f) thiepin-5,5-dioxide (L636,499) and (IS-(1 α ,2 β (5Z),3 β ,4 α))-7-(3-((2-((phenylamino)-carbonyl)hydrazino)methyl)-7-oxabicyclo(2.2.1)-hept-2-yl)-5-heptenoic acid (SQ 29,548), were studied to ensure that the effects seen in vivo were mediated by receptor antagonism and did not reflect a nonspecific drug effect. Both compounds specifically inhibited platelet aggregation induced by arachidonic acid and by the prostaglandin endoperoxide analogue, U46619, in vitro and ex vivo, and increased the time to thrombotic vascular occlusion in vivo. When an antagonist (L636,499) was administered at the time of occlusion in vehicle-treated dogs, coronary blood flow was restored. In vitro L636,499 and a third antagonist, 13-azaprostanic acid, specifically reversed endoperoxide-induced platelet aggregation and vascular smooth muscle contraction. Neither compound altered cyclic AMP in platelet-rich plasma before or during disaggregation. Therefore, reversal of coronary occlusion may reflect disaggregation of platelets and/or relaxation of vascular smooth muscle at the site of thrombus formation through specific antagonism of the thromboxane A₂/prostaglandin endoperoxide receptor. Thromboxane A₂/prostaglandin endoperoxide receptor antagonists are compounds with therapeutic potential which represent a novel approach to defining the importance of thromboxane A₂ and/or endoperoxide formation in vivo.

Introduction

Thromboxane A₂ is the major metabolite of arachidonic acid in platelets formed via cyclic prostaglandin endoperoxides by the enzymes cyclooxygenase and thromboxane synthase (1). In vitro, thromboxane A₂ induces vascular smooth muscle contraction and platelet aggregation (1). However, its evanescent nature has precluded direct study in vivo, so that its putative role in vascular occlusion is based largely on indirect evidence using inhibitors of the thromboxane A₂ synthase and cyclooxygenase enzymes (2). Interpretation of these studies has been

complicated by the nonspecific action of these agents (3-6). More recently, several compounds have been described that antagonize the effects of thromboxane A₂ and prostaglandin endoperoxides at the common receptor site for these proaggregatory vasoconstrictor compounds (7-16). A theoretical limitation to using such compounds as antithrombotic agents in vivo is that platelet agonists such as collagen may permit continued thromboxane independent platelet activation despite efficient receptor blockade. To elucidate the role of thromboxane A₂/prostaglandin endoperoxide receptor activation during platelet-dependent vascular occlusion, we have studied two structurally distinct receptor antagonists, 3-carboxyl-dibenzo (b,f)thiepin-5,5-dioxide (L636,499)¹ (13, 14) and (IS-(1 α ,2 β (5Z),3 β ,4 α))-7-(3-((2-((phenylamino)-carbonyl)hydrazino)methyl)-7-oxabicyclo(2.2.1)-hept-2-yl)-5-heptenoic acid (SQ 29,548) (16) in a canine model of coronary vascular thrombosis.

Methods

Animal experiments. The model used was a modification of that described by Luchessi and co-workers (17). Mongrel dogs (18-32 kg) were anesthetized with sodium pentobarbital (30 mg/kg plus a continuous infusion intravenously) and ventilated with room air by positive pressure ventilation (Harvard respirator pump; Harvard Apparatus Co., Inc., S. Natick, MA). The heart was suspended in a pericardial cradle through a left thoracotomy in the fifth intercostal space and the left circumflex coronary artery was isolated proximal to the first marginal branch. An electrode was passed through the wall of the left circumflex coronary artery so that 4-5 mm of exposed tip lay against the endothelial surface. The electrode consisted of 30-gauge silver-coated copper wire insulated with Teflon onto which was crimped the tip of a 28-gauge hypodermic needle to aid passage of the electrode through the vessel wall. The electrode was connected in series with a 9-V battery, an ammeter, a 20,000- Ω resistor, and a 50,000- Ω potentiometer, and the circuit completed by grounding to the subcutaneous tissues (Fig. 1). Arterial blood pressure was recorded through a polyethylene catheter in the femoral artery and, in some cases, through a 23-gauge needle in the left circumflex coronary artery distal to the electrode. Left circumflex coronary artery blood flow was recorded by a 2.0-3.0-mm electromagnetic flow probe (Gould Statham Instruments, Puerto Rico) positioned proximal to the electrode site. The femoral and jugular veins were cannulated for drug administration and blood withdrawal. All recordings were made simultaneously on a 6-channel physiological recorder (HP 1758A). After a 30-min stabilization period, L636,499 (10-30 mg/kg) or vehicle (NA₂CO₃ 0.05 M) were administered intravenously over 10 min followed by a 10-min equilibration period before initiation of a 150- μ A current. Blood was withdrawn prior to, at 1 h after drug administration, and upon completion of the study for platelet aggregation studies for measurement of serum thromboxane B₂ and serum drug concentrations. In a separate series of experiments, SQ 29,548 (0.2 mg/kg) or vehicle (normal saline) was administered intravenously before initiation of a 200- μ A current followed by an infusion

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1. *Abbreviations used in this paper:* 13-APA, 13-azaprostanic acid; L636,499, 3-carboxyl-dibenzo (b,f)thiepin-5,5-dioxide; SQ 29,548, (IS-(1 α ,2 β (5Z),3 β ,4 α))-7-(3-((2-((phenylamino)-carbonyl)-hydrazino)methyl)-7-oxabicyclo(2.2.1)-hept-2-yl)-5-heptenoic acid.

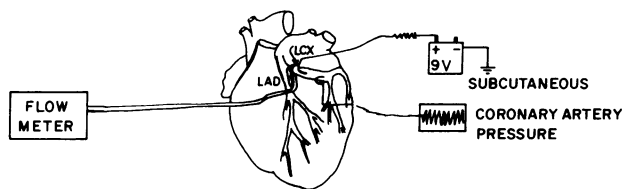


Figure 1. Model of platelet-dependent occlusion of the left circumflex coronary artery (LCX) in the dog after electrically induced endothelial injury. LAD, left anterior descending coronary artery.

of SQ 29,548 (0.2 mg/kg per h). This protocol was based on the biological half-life of the drug determined in prior experiments. Blood was withdrawn before and at 1 h after drug administration for platelet aggregation studies and serum thromboxane B₂ determination.

Platelet studies. Platelet aggregation was assessed *in vitro* and *ex vivo* at 37°C in platelet-rich plasma by light transmission (18) using a four-channel aggregometer (model Pap-4; BioData Corp., Hartboro, PA). Platelet disaggregation was also assessed in platelet-rich plasma by light transmission and in whole blood by impedance aggregometry (Chronolog Corp., Havertown, PA) which records changes in electrical impedance between two platinum electrodes induced by adhering platelet aggregates (19). Platelet-rich plasma was prepared by centrifuging citrated (9:1, vol/vol) whole blood at 200 g for 10 min; and was diluted to 300,000 platelets/ml by autologous platelet-poor plasma. As canine platelets from citrated whole blood aggregate poorly in response to arachidonic acid or prostaglandin endoperoxides (20), they were first primed with a subthreshold concentration of ADP (0.5–2.0 μM), which induced a small reversible primary wave of aggregation before addition of the agonist. The threshold concentration of each agonist, that is, the lowest concentration of each agonist inducing maximal (80–95%) platelet aggregation, was determined. Platelet aggregation to threshold concentrations of arachidonic acid (Sigma Chemical Co., St. Louis, MO) (0.17–0.33 μM), (15S)-hydroxy 11,9-(epoxymethano)-prostadienoic acid (U46619) (0.67–2.5 μM), and ADP (5–10 μM) (Sigma Chemical Co.) was assessed before and after incubation with the thromboxane A₂/prostaglandin endoperoxide receptor antagonists, L636,499, SQ 29,548, and 13-azaprostanoic acid (13-APA) (15) for 10 min or after administration of the antagonists in animal experiments. Disaggregation studies were performed in aspirinated (10⁻⁴ M) canine platelet-rich plasma and whole blood aggregated with U46619 or ADP by the addition of vehicle or drug at different time intervals after addition of the agonist.

Vascular smooth muscle studies. Spiral arterial strips were prepared from freshly isolated canine coronary arteries and rat aortae in oxygenated modified Krebs's solution at 4°C. The vascular strips were suspended in oxygenated Krebs's solution (pH 7.35) at 37°C in a 10-ml tissue bath (Phipps and Bird, Richmond, VA). Tension was recorded with a force displacement transducer (FT03, Grass Instruments, Quincy, MA) connected to a polygraph (model 79 D; Grass Instruments). Tension was gradually increased over a 45-min period to a resting tension of 1.5–2.0 g during which the bathing fluid was repeatedly replaced. Agonist dose-response curves were determined and resting tension reestablished by multiple washings over 45 min before addition of the thromboxane A₂/prostaglandin endoperoxide receptor antagonist.

Biochemical studies. Serum thromboxane B₂ was determined in whole blood incubated at 37°C for 45 min by radioimmunoassay (21). Serum concentrations of L636,499 were determined by high performance liquid chromatography (μ-Bondpak C-18 reverse phase column in series with a spectrophotometer [model 480 LC; Waters Associates, Millipore Corp., Milford, MA]). The solvent system was 65:35 phosphate buffer, pH 3.5: acetonitrile, and ultraviolet detection was set at 310 nm. The internal standard used was the 9-fluoro analogue of L636,499. A standard curve was obtained by spiking normal dog serum with known amounts of L636,499 and internal standard, and submitting these samples to the same analytical procedure. Comparison of the integrated values obtained with the samples and the standard curve was used to determine the concentration of L636,499 in the unknown samples.

Cyclic AMP was determined in platelet-rich plasma by radioimmunoassay as described by Steiner et al. (22) and modified by Harper and Brooker (23) following the extraction procedure of Jacobs et al. (24).

Data analysis. All data are expressed as the mean ± SEM. Unpaired data were compared by the Wilcoxon rank sum test and paired data by a modification of Student's paired *t* test for small sample size (25).

Results

Animal studies. Passage of a 150-μA current through the circuit resulted in vessel occlusion in 1–2 h. On light microscopy, the occlusive thrombus consisted of a homogenous material with entrapped red blood cells. This material failed to stain for fibrin and was found to be largely composed of platelets on electron microscopy (Fig. 2). Treatment with the thromboxane A₂/prostaglandin endoperoxide receptor antagonist L636,499 intravenously, before passage of a 150-μA current through the electrode, delayed the time to occlusion in a dose-dependent manner (Fig. 3). At 30 mg/kg of L636,499, the time to occlusion was 290 ± 43 min (*n* = 7; mean ± SEM) compared with 92 ± 12 min for vehicle-treated controls (*n* = 14; *P* < 0.01). The mean time to occlusion in treated dogs includes four dogs in whom occlusion failed to occur during 6 h of observation and who were assigned an occlusion time of 6 h. Similarly, treatment with 0.2 mg/kg SQ 29,548 plus 0.2 mg/kg per h intravenously throughout the duration of the experiment delayed the time to occlusion (229 ± 5.9 min, *n* = 3) compared with vehicle-treated controls (50 ± 22.0 min, *n* = 3). One SQ 29,548-treated dog failed to occlude during 4 h of observation and was assigned an occlusion time of 4 h. The shorter time to occlusion in SQ 29,548-treated dogs and their respective vehicle-treated controls reflects the higher current (200 μA) used in these experiments. There was no difference in arterial blood pressure or coronary blood flow between drug- and vehicle-treated dogs.

In 5 control dogs, 30 mg/kg L636,499 administered intravenously 2 min after complete occlusion of the left circumflex coronary artery resulted in reperfusion (>80% of control coronary blood flow) in all cases. In two dogs, coronary flow was maintained during 1 h of subsequent observation during which current flow was continued (Fig. 4), whereas in the remaining three dogs reocclusion occurred in <30 min. In contrast, administration of vehicle (*n* = 3) had no effect (Fig. 4).

Platelet studies. *In vitro* experiments demonstrated that all three thromboxane A₂/prostaglandin endoperoxide receptor antagonists, L636,499, SQ 29,548, and 13-APA, inhibited platelet aggregation induced by threshold concentrations of arachidonic acid and U46619 but not by ADP. L636,499 and 13-APA were almost equipotent; maximum inhibition of the response to the threshold concentration of U46619 occurred at 2 × 10⁻⁴ M (Fig. 5). By contrast, SQ 29,548 was more potent, exhibiting a similar effect at 10⁻⁷ M.

Inhibition of coronary occlusion by L636,499 was associated with almost complete inhibition of platelet aggregation to the threshold concentration of U46619 but not to ADP *ex vivo* in all but one dog at 1 h after drug administration (Fig. 6). Similarly, platelet aggregation to the threshold concentration of arachidonic acid was markedly inhibited (92 ± 6%). Corresponding serum levels of L636,499 were 96.4 ± 7.2 μg/ml (3 × 10⁻⁴ M), which confirmed adequate bioavailability for a thromboxane A₂/prostaglandin endoperoxide-mediated effect *in vivo*. Platelet aggregation to U46619 had at least partially recovered at the time of occlusion or at the end of the observation period. Thus, the degree of inhibition of U46619-induced platelet aggregation was

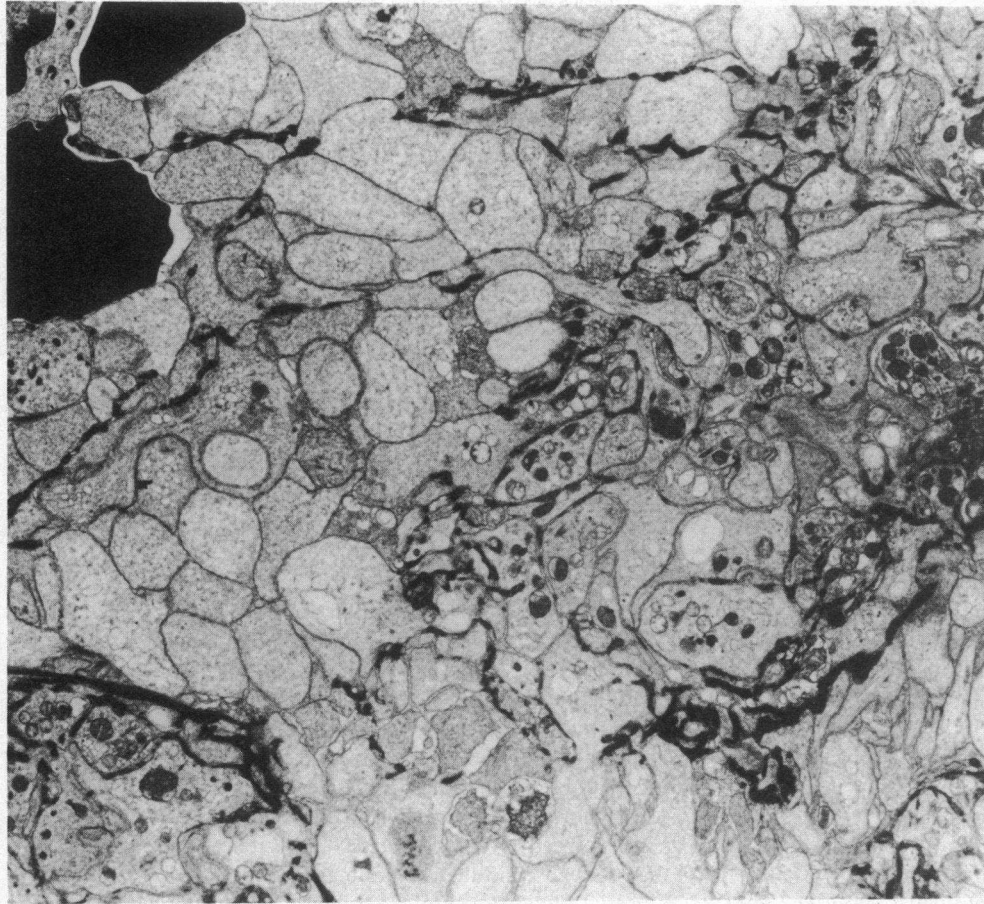


Figure 2. Transmission electron micrograph of the thrombus obtained from the left circumflex coronary artery after electrically induced occlusion in the dog. The section demonstrates the predominance of platelets that show evidence of activation including degranulation and interdigititation.

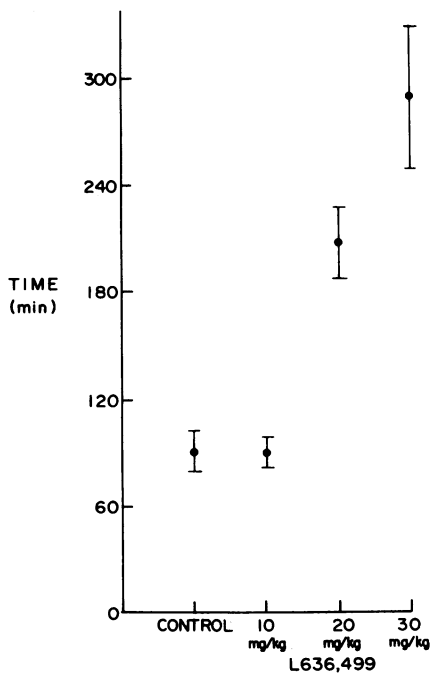


Figure 3. Dose-dependent relationship of the effect of L636,499 on the time to coronary occlusion in dogs after electrically induced ($150 \mu\text{A}$) endothelial injury (mean \pm SEM). The occlusion time at peak dose is the mean of 7 dogs, 4 of whom failed to occlude during 6 h of observation and were assigned a value of 6 h. At all other doses an absolute occlusion time was obtained in all dogs.

$59 \pm 15\%$ at the time of occlusion compared with $82 \pm 12\%$ at 1 h after initiation of the current. Similarly, platelet aggregation in response to threshold concentrations of arachidonic acid and U46619, but not to ADP, was completely inhibited ex vivo after administration of SQ 29,548 in all dogs (Fig. 6). Serum thromboxane B_2 decreased by $27 \pm 9.2\%$ at 1 h following administration of L636,499, but was unaltered in control animals. Although no significant change in serum thromboxane B_2 occurred after SQ 29,548 (Fig. 6), this may reflect the small sample size.

Possible mechanisms for the reinstatement of coronary flow by L636,499 following occlusion in this model include disaggregation of platelets within the thrombus and relaxation of vascular smooth muscle contracted by thromboxane A_2 which has been released locally at the occlusion site. To address the first mechanism, L636,499 was added to aspirinated (10^{-4} M) canine platelets aggregated with U46619 (1.25 – $2.5 \mu\text{M}$) or with ADP (10 – $20 \mu\text{M}$) in vitro. L636,499 caused a dose- and time-dependent disaggregation of canine platelets aggregated with U46619 (Fig. 7) with all dogs ($n = 7$) demonstrating the effect at 7×10^{-4} M L636,499, but had no effect on ADP-induced platelet aggregation. Disaggregation was also demonstrated in whole blood after platelet aggregation with U46619. A similar effect was demonstrated using a structurally distinct thromboxane A_2 /prostaglandin endoperoxide receptor antagonist, 13-APA. Cyclic AMP concentration was determined in platelet-rich plasma before and during platelet aggregation induced by U46619, and during subsequent disaggregation induced by L636,499 and 13-APA; two determinations were made during each phase. Cyclic AMP remained unaltered during U46619-induced platelet ag-

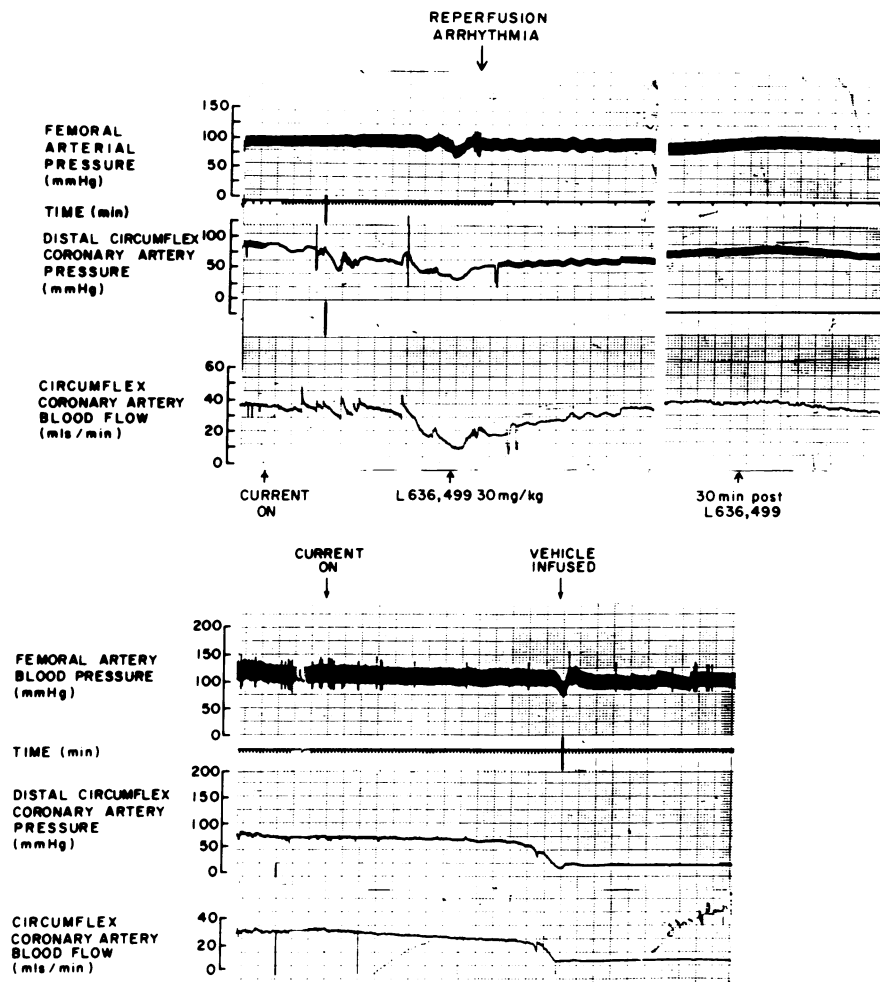


Figure 4. Comparison of the effects of L636,499 30 mg/kg (top) and vehicle (bottom) administered 2 min after coronary occlusion. After initiation of current, coronary occlusion occurs in ~1 h as indicated by a reduction in coronary flow and coronary artery pressure distal to the electrode site. L636,499 infused at the time of occlusion induced reperfusion which was associated with an arrhythmia, whereas vehicle was without effect.

gregation (15.1 ± 2.4 and 13.9 ± 2.0 fmol/ μ l) and subsequent disaggregation induced by L636,499 (12.9 ± 6.2 and 16.7 ± 2.4 fmol/ μ l) compared with baseline (11.8 ± 1.8 and 22.2 ± 6.2 fmol/ μ l) ($n = 5$). Similarly, disaggregation induced by 13-APA was unassociated with an increase in cyclic AMP in platelet-rich plasma.

Vascular smooth muscle studies. To address the possibility that relaxation of vascular smooth muscle at the occlusion site might be the mechanism of reperfusion induced by thromboxane

A_2 /prostaglandin endoperoxide receptor antagonism in this model, the effects of L636,499 and 13 APA on arterial strips were studied in vitro. L636,499 (10^{-5} M and 3×10^{-5} M) inhibited U46619-induced vascular smooth muscle contraction, increasing the EC_{50} of U46619 by a factor of 17 and 50, respectively (Fig. 8), whereas the responses to norepinephrine and potassium were unaltered. Both L636,499 (2.5 – 5×10^{-5} M) and

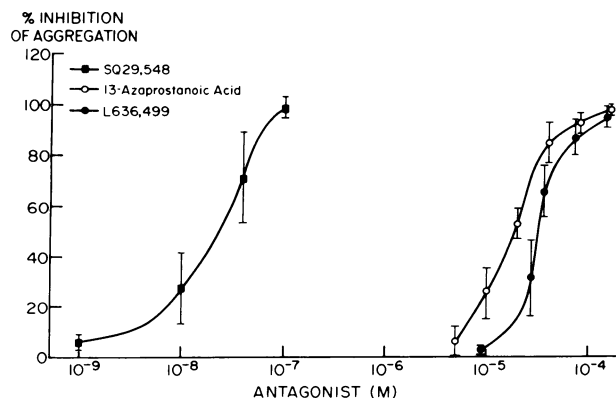


Figure 5. Inhibition by L636,499 ($n = 5$), SQ 29,548 ($n = 3$), and 13-APA ($n = 3$) of platelet aggregation induced by threshold concentrations of U46619 in vitro.

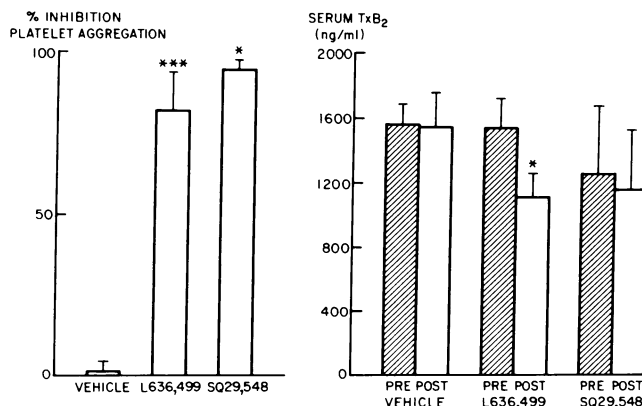


Figure 6. Effect of vehicle ($n = 14$), L636,499 ($n = 7$), and SQ 29,548 ($n = 3$) on U46619-induced platelet aggregation and serum thromboxane B_2 (TxB_2) ex vivo 1 h after initiation of current. (* $P < 0.05$; *** $P < 0.001$ vs. pretreatment).

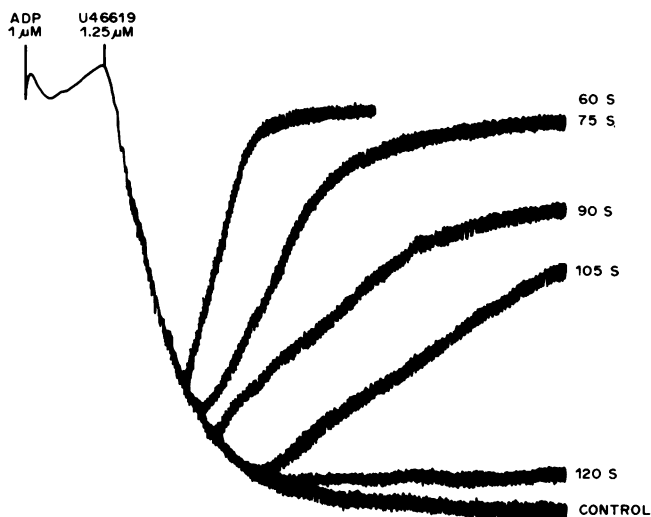


Figure 7. Disaggregation of aspirinated (10^{-4} M) platelets in vitro by L636,499 after aggregation with U46619. Platelet-rich plasma was incubated in 10^{-4} M aspirin for 10 min. Following priming with ADP ($1 \mu\text{M}$), U46619 ($1.25 \mu\text{M}$) was added to the cuvette. The figure shows a series of experiments superimposed in which L636,499 was added to the cuvette at the time intervals shown and resulted in disaggregation of platelets. Platelet aggregation and disaggregation were monitored by light transmission.

13-APA ($3-5 \times 10^{-5}$ M) induced relaxation of vascular smooth muscle precontracted with U46619 ($0.63-1.25 \mu\text{M}$), but had no effect on strips precontracted with norepinephrine (10^{-6} M) (Fig. 9).

Discussion

These studies demonstrate the efficacy of thromboxane A_2 /prostaglandin endoperoxide receptor antagonists in vivo in a model of thrombotic occlusion. Thus, they provide evidence for thromboxane A_2 /endoperoxide receptor activation as a predominant mechanism in this model of platelet-dependent coronary occlusion. This conclusion is based on the platelet de-

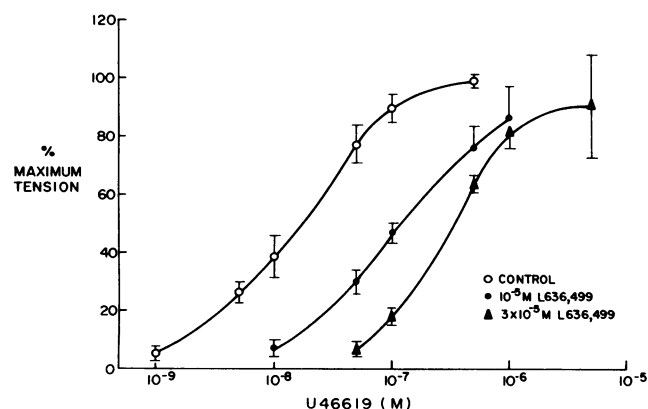


Figure 8. Inhibition of U46619-induced vascular smooth muscle contraction by L636,499. Vascular smooth muscle spiral strips were prepared from fresh canine coronary arteries and suspended in oxygenated (95% O_2 , 5% CO_2) Krebs' solution. Cumulative dose-response curves to U46619 were determined before and after 10 min incubation with L636,499 ($n = 5$).

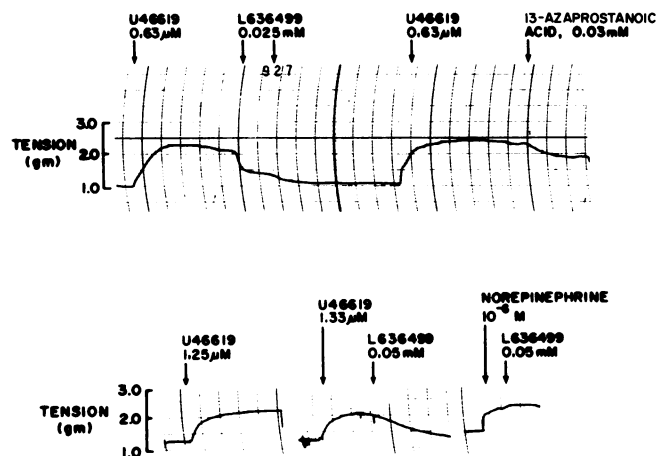


Figure 9. Relaxation of canine coronary (top) and rat aorta (bottom) spiral strips induced by L636,499 and 13-APA after contraction with U46619 but not norepinephrine.

pendency of the canine model used and on the pharmacological specificity of the compounds studied. The role of platelet activation in this model is illustrated by the morphological appearance of the occlusive material and by studies demonstrating inhibition of occlusion by prostacyclin but not heparin (17, 26). Specificity of the pharmacological effects of L636,499 and SQ 29,548 was demonstrated in vitro and ex vivo. Thus, both compounds specifically inhibited platelet activation at the level of the common thromboxane A_2 /prostaglandin endoperoxide receptor. L636,499 is a specific antagonist of the platelet thromboxane A_2 /prostaglandin endoperoxide receptor at the concentrations attained in vivo, although it has weak antiserotonergic properties at higher concentrations (13, 14). In addition, SQ 29,548 has no serotonin antagonist activity (16). Consistent with their effects as thromboxane A_2 /prostaglandin endoperoxide receptor antagonists, these compounds did not increase cyclic AMP generation in platelet-rich plasma, as occurs with phosphodiesterase inhibitors and prostacyclin. Similarly, it is unlikely that these compounds mediate their effects through increased cyclic GMP generation since this would result in nonspecific inhibition of platelet aggregation and relaxation of vascular smooth muscle (27, 28), whereas the inhibitory effects of the compounds studied were specific for endoperoxide. Although serum thromboxane B_2 was decreased by L636,499 ex vivo, the decrease was slight relative to the degree of inhibition of arachidonate-induced platelet aggregation and probably reflects inhibition of platelet aggregation, and subsequent thromboxane A_2 generation, by thromboxane A_2 /prostaglandin endoperoxide receptor antagonism during whole blood clotting. This is supported by the finding that L636,499 does not effect thromboxane A_2 generation by platelet microsomes (13) and that a similar effect has been demonstrated with other structurally distinct thromboxane A_2 /prostaglandin endoperoxide receptor antagonists (8, 29). Thus, there is no evidence that these compounds directly inhibit the thromboxane A_2 synthase enzyme. Consistent with their effects in vitro, both L636,499 and SQ 29,548 demonstrated specific inhibition of thromboxane A_2 /prostaglandin endoperoxide-induced platelet aggregation ex vivo after administration in the dog. These findings suggest that the in vivo effects of L636,499 and SQ 29,548 are mediated by specific thromboxane A_2 /prostaglandin endoperoxide receptor antagonism and are not reflec-

tive of some nonspecific activity. This is further supported by the dose dependency of the in vivo response to L636,499, the finding that the plasma concentrations of L636,499 achieved were within the range in which this compound acts as a thromboxane A₂/prostaglandin endoperoxide receptor antagonist, and that a similar effect was demonstrated in vivo with two structurally distinct compounds with similar pharmacological profiles. Subsequent studies with L636,499 in this model using the higher current of 200 μ A have confirmed these findings. L636,499 at a dose of 20 mg/kg followed by an infusion of 2 mg/kg per min increased the time to occlusion by 84 \pm 26% compared with vehicle-treated controls (Fitzgerald, D. J., J. Frassetta, and G. A. Fitzgerald, unpublished data).

An important additional finding of this study was that coronary occlusion could be reversed by thromboxane A₂/prostaglandin endoperoxide receptor antagonism. The possible mechanisms, supported by in vitro experiments, were disaggregation of platelet aggregates and relaxation of vascular smooth muscle contracted at the occlusion site by locally generated thromboxane A₂. Disaggregation of canine platelets in vitro by L636,499 was specifically due to antagonism of the thromboxane A₂/prostaglandin endoperoxide receptor, since disaggregation occurred only in platelets aggregated with a prostaglandin endoperoxide analogue and was also demonstrated using a structurally distinct thromboxane A₂/prostaglandin endoperoxide receptor antagonist, 13-APA. Consistent with this hypothesis, neither L636,499 nor 13-APA increased cyclic AMP in platelet-rich plasma, in contrast to prostacyclin, which also causes platelet disaggregation (30). Previous studies have demonstrated that 13-APA reverses arachidonic acid (31) and U46619-induced aggregation (32) of human platelets without altering platelet cyclic AMP (32), and like U46619, displaces [³H]13-APA from isolated platelet membranes (33). These observations suggest that continued occupancy of the thromboxane A₂/prostaglandin endoperoxide receptor is required during the early phase of thromboxane A₂-induced platelet activation for complete, irreversible aggregation to occur. A similar mechanism operating in vivo may explain the reperfusion seen with L636,499, although a role for endogenous platelet inhibitory prostaglandins, such as PGI₂, in mediating platelet disaggregation in vivo cannot be ruled out. Another possible mechanism is relaxation of vascular smooth muscle contracted at the occlusion site by locally released thromboxane A₂ or prostaglandin endoperoxides. L636,499 and 13-APA (32) are antagonists of contractile prostanoids on vascular smooth muscle, and we have demonstrated that these two structurally distinct thromboxane A₂/prostaglandin endoperoxide receptor antagonists relax vascular smooth muscle contracted in vitro by U46619 but not by epinephrine or potassium.

Despite the lack of a direct response of canine platelets to thromboxane or prostaglandin endoperoxides in up to 70% of dogs (20), these studies demonstrate an important functional role for thromboxane in this species. This is consistent with studies demonstrating effectiveness of cyclooxygenase inhibitors in models of platelet-dependent vasoocclusion in dogs (17, 35–38). Canine platelets generate thromboxane, and thromboxane A₂/prostaglandin endoperoxide receptors have been demonstrated in canine platelet preparations (39). Priming of canine platelets in vitro has been shown not to alter either receptor density or affinity (39), so that the lack of a direct response to thromboxane appears to be a post-receptor event and may be an in vitro artifact. The presence of a platelet thromboxane A₂/prostaglandin endoperoxide receptor, the ability of canine plate-

lets to generate thromboxane, and the functional importance of thromboxane in the canine are clearly defined and make this an appropriate species for these types of studies.

In conclusion, these studies demonstrate the efficacy and specificity of two thromboxane A₂/prostaglandin endoperoxide receptor antagonists in vitro and in vivo and provide evidence that thromboxane A₂/endoperoxide receptor activation is the predominant mechanism underlying the coronary occlusion which occurs in this model. Histologic evidence of platelet thrombi in the coronary arteries of patients who suffered sudden death (40), the efficacy of aspirin in unstable angina (41), and our recent demonstration that thromboxane A₂ biosynthesis increases coincident with chest pain in patients with unstable angina (42) also implicate thromboxane-dependent platelet activation in human syndromes of coronary vascular occlusion. We conclude that thromboxane A₂/prostaglandin endoperoxide receptor antagonists promise to be selective probes in further clarifying the role of thromboxane A₂ formation in vivo and represent a novel approach to the treatment of vasoocclusive disease in man.

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