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Research Article

Prostaglandin endoperoxides (PGG2/PGH2), precursors of thromboxane (TX) A2 and prostaglandins, may accumulate sufficiently in the presence of a TXA2 synthase inhibitor to exert biological activity. To address whether this modulates the response to TXA2 synthase inhibition in the setting of thrombosis in vivo, we examined the interaction of a TXA2 synthase inhibitor (U63,557a) and a TXA2/prostaglandin endoperoxide receptor antagonist (L636,499) in a canine model of coronary thrombosis after electrically induced endothelial injury. U63,557a exerted little inhibitory effect in this model despite a marked reduction in serum TXB2 and urinary 2,3-dinor-TXB2, an index of TXA2 biosynthesis. Combination of the two drugs was more effective than either drug alone. The enhanced effect achieved upon addition of the TXA2/prostaglandin endoperoxide receptor antagonist to the TXA2 synthase inhibitor suggests that the response to the latter compound was limited by the proaggregatory effects of prostaglandin endoperoxides. The increased effect of the combination over the receptor antagonist alone may reflect metabolism of PGG2/PGH2 to platelet inhibitory prostaglandins. This is supported by the following findings: (a) urinary 2,3-dinor-6-keto-PGF1 alpha, an index of prostacyclin biosynthesis, increased after administration of the synthase inhibitor, an effect that was exaggerated in the presence of thrombosis; (b) inhibition of arachidonate-induced platelet aggregation by U63,557a was dependent on the formation of a platelet-inhibitory prostaglandin; and (c) pretreatment with aspirin abolished the synergism between […]

Prostaglandin Endoperoxides Modulate the Response to Thromboxane Synthase Inhibition during Coronary Thrombosis

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Abstract

Prostaglandin endoperoxides (PGG_2/PGH_2), precursors of thromboxane (TX) A_2 and prostaglandins, may accumulate sufficiently in the presence of a TXA_2 synthase inhibitor to exert biological activity. To address whether this modulates the response to TXA_2 synthase inhibition in the setting of thrombosis in vivo, we examined the interaction of a TXA_2 synthase inhibitor (U63,557a) and a TXA_2/prostaglandin endoperoxide receptor antagonist (L636,499) in a canine model of coronary thrombosis after electrically induced endothelial injury. U63,557a exerted little inhibitory effect in this model despite a marked reduction in serum TXB_2 and urinary 2,3-dinor-TXB_2, an index of TXA_2 biosynthesis. Combination of the two drugs was more effective than either drug alone. The enhanced effect achieved upon addition of the TXA_2/prostaglandin endoperoxide receptor antagonist to the TXA_2 synthase inhibitor suggests that the response to the latter compound was limited by the proaggregatory effects of prostaglandin endoperoxides. The increased effect of the combination over the receptor antagonist alone may reflect metabolism of PGG_2/PGH_2 to platelet inhibitory prostaglandins. This is supported by the following findings: (*a*) urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$, an index of prostacyclin biosynthesis, increased after administration of the synthase inhibitor, an effect that was exaggerated in the presence of thrombosis; (*b*) inhibition of arachidonate-induced platelet aggregation by U63,557a was dependent on the formation of a platelet-inhibitory prostaglandin; and (*c*) pretreatment with aspirin abolished the synergism between these compounds. These studies demonstrate that prostaglandin endoperoxides modulate the response to TXA_2 synthase inhibition in vivo and identify a drug combination of potential therapeutic efficacy in the prevention of thrombosis.

Introduction

Thromboxane (TX) A_2, the major cyclooxygenase product of arachidonic acid in platelets, is a potent platelet aggregant and vasoconstrictor (1) and plays a pathogenic role in a variety of ischemic heart disease syndromes (2–6). A reduction in TXA_2 biosynthesis may be achieved by inhibition of TXA_2 synthase, the enzyme that catalyses the conversion of prostaglandin endoperoxides (PGG_2/PGH_2) to TXA_2 (7–9). This has a theoretical advantage over cyclooxygenase inhibition, most commonly achieved with aspirin, in that it preserves prostacyclin (PGI_2) biosynthesis (7–9). PGI_2 is a major cyclooxygenase product of arachidonic acid in vascular endothelium and is a potent platelet inhibitor and vasodilator (10). Evidence from experimental studies suggests that PGI_2 limits platelet activation in vivo (11, 12). Furthermore, PGI_2 biosynthesis is increased in conditions associated with platelet activation, including severe atherosclerosis (13), unstable angina (2), and systemic sclerosis (14). Coincident inhibition of PGI_2 formation, therefore, may limit the therapeutic response to aspirin and other cyclooxygenase inhibitors. In contrast, TXA_2 synthase inhibitors do not inhibit PGI_2 biosynthesis (7–9).

Despite their biochemical selectivity, TXA_2 synthase inhibitors are weak platelet inhibitors in vitro (7, 15–17) and exert variable effects in vivo (18–21). This may reflect the continued formation of platelet prostaglandin endoperoxides that activate a receptor, shared with TXA_2, mediating platelet activation and vasoconstriction (7, 15, 17). Indeed, the antiplatelet effects of TXA_2 synthase inhibitors may be largely mediated through platelet-inhibitory prostaglandins formed by the metabolism of accumulated prostaglandin endoperoxides. Marcus et al., and Schafer et al., have demonstrated that, in the presence of a TXA_2 synthase inhibitor, platelet-derived prostaglandin endoperoxides may be metabolized by contiguous endothelium to PGI_2 (22, 23). Other products, including PGE_2 and PGD_2, may also be formed and mediate the platelet effects of TXA_2 synthase inhibitors (24, 25). Pharmacologic studies have also implied a role for vascular-derived prostaglandins in mediating the response to TXA_2 synthase inhibition in vivo (11, 26). Thus, prostaglandin endoperoxides may modulate the response to TXA_2 synthase inhibitors, both by substituting for the proaggregatory effects of TXA_2 and through their metabolism to platelet-inhibitory prostaglandins.

To determine if these mechanisms influence thrombogenesis in vivo, we have examined the interaction of a TXA_2 synthase inhibitor and an antagonist of the shared TXA_2/prostaglandin endoperoxide receptor in a canine model of coronary artery thrombosis (27, 28). These studies demonstrate biochemical and functional evidence of a role for prostaglandin endoperoxides in modulating the response to TXA_2 synthase inhibitors in vivo and identify an interaction of potential therapeutic importance between these classes of drugs.

Methods

Animal studies

Acute experiments. All animal studies were reviewed and approved by the Animal Care Committee at Vanderbilt University. Male mongrel dogs (17–23 kg) were anesthetized with pentobarbitone 30 mg/kg and ventilated using a Harvard respirator (Harvard Apparatus Co., Natick, MA). The circumflex coronary artery was isolated through a left thoracotomy and all branches ligated down to the first obtuse marginal branch. A needle electrode was passed through the arterial wall such that 4–5 mm of exposed wire lay against the endothelium, as pre-

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viously described (27, 28). The electrode consisted of 30-gauge Teflon coated silver wire onto the end of which was crimped the tip (2 mm) of a 28-gauge hypodermic needle to aid passage through the vessel wall. The electrode was connected in series with a 50,000 Ω potentiometer, a 20,000 Ω resistor, an ammeter and the positive terminal of a 9-V battery, and the circuit was grounded to the subcutaneous tissues of the dog. An electromagnetic flow probe (Carolina Instruments, King, NC) was positioned proximal to the electrode site and connected to an electromagnetic flowmeter (SP 2202; Gould-Statham Instrument Co., Inc., Hato Rey, Puerto Rico). Blood pressure was recorded through a polyethylene catheter implanted in the femoral artery. Coronary blood flow and femoral artery pressure were recorded continuously throughout the experiment using a strip chart recorder (HP 1758A; Hewlett Packard Co., Waltham, MA).

Animals were randomized to one of four treatments. Group I animals received vehicle only and acted as controls. In group II, the TXA_2 synthase inhibitor, 5-(3'-pyridinylmethyl) benzfuran-2-carboxylate (U63,557a), was administered in a bolus dose of 10 mg/kg i.v. in normal saline over 10 min. In group III, the TXA_2/prostaglandin endoperoxide receptor antagonist, 3-carboxyl-dibenzo (b,f) thiepin-5,5-dioxide (L636,499), was administered in a bolus dose of 20 mg/kg followed by 2 mg/kg per min. In group IV, both U63,557a and L636,499 were administered in these doses.

After a stabilization period of 30 min, drug or vehicle was administered. After a further 30 min coronary thrombosis was induced by passing a 150–200 μA current through the electrode and the animal observed for 4 h or until complete coronary occlusion had occurred. The time to coronary occlusion, defined as the time from the onset of current to the point at which zero flow was achieved, was compared in the four groups. Blood was obtained before and hourly following drug administration for platelet aggregation studies and for measurement of serum TXB_2.

Chronic experiments. In chronic studies, the same surgical procedure was used with the exception that an ultrasonic flow probe (Biomedical Engineering, Iowa University, Iowa City, IA) was implanted in place of the electromagnetic flow probe. The terminals of the flow probe and the electrode were brought to the surface in a subcutaneous pouch, the chest closed and the animal allowed to recover. Heparin (100 IU/kg s.c.) was administered every 8 h for 48 h. 5 d after surgery, the animal was sedated with acepromazine 1 mg/kg and morphine sulfate 1-2 mg/kg. The electrode terminal was recovered and connected to the circuit described above. The terminals of the ultrasonic flow probe were connected to a directional pulsed Doppler flowmeter (545C-4, Biomedical Engineering, Iowa University). Arterial blood pressure was not recorded in these experiments to avoid traumatic increases in eicosanoid biosynthesis.

Animals were randomized to the same study groups as in the acute experiments. An additional group (group V) of animals received aspirin 20 mg/kg in 0.1 M Na_2CO_3 i.v. over 10 min before the administration of the combination of U63,557a and L636,499. Coronary thrombosis was induced by passing a 200 μA current through the electrode and the animal observed for a maximum of 7 h or until 2 h after complete coronary occlusion. Urine was collected by catheterization before drug administration and hourly thereafter for measurement of 2,3-dinor-TXB_2 and 2,3-dinor-6-keto-prostaglandin (PG) $F_{1\alpha}$, major urinary metabolites of TXA_2 and PGI_2, respectively (29, 30).

Platelet aggregation studies

Platelet aggregation to arachidonic acid (0.075–0.66 mM) (Sigma Chemical Co., St. Louis, MO) and to the endoperoxide analogue, (15S)-hydroxy 11,9-(epoxymethano)-prostadienoic acid (U46619, 0.13–2.0 μM) (Upjohn Co., Kalamazoo, MI) was determined in platelet-rich plasma (PRP) by light transmission (31), using a multichannel aggregometer (Biodata PAP-4, Biodata, Hartboro, PA). PRP was prepared by centrifuging citrated venous blood (3.8% Na citrate, 9:1 vol/vol) at 3,000 rpm for 50 s and adjusted to 300,000 platelets/μl with platelet-poor plasma obtained by centrifuging the remaining blood at 3,000 rpm for 10 min. Platelet aggregations were performed in 500-μl aliquots of plasma with aggregating agents added in volumes of 10% or less. The concentration of aggregating agent is expressed as the final concentration in PRP. As canine platelets may fail to aggregate directly to arachidonic acid and U46619 in 30–50% of cases, the platelets were first primed with ADP at a concentration (1–2 μM) that induced a small reversible wave of aggregation, as previously described (32).

Biochemical studies

Urinary 2,3-dinor TXB_2 and 2,3-dinor-6-keto $PGF_{1\alpha}$ were determined by gas chromatography, negative ion-chemical ionization, mass spectrometry using their respective tetradeuterated analogues as internal standards (30, 33). Briefly, to a 5-ml sample of urine was added 5 ng of each internal standard and the urine extracted by immunoaffinity chromatography as previously described (34). After formation of the methoxime derivatives, further purification was achieved by thin-layer chromatography and the sample finally derivatized to the trimethylsilyl ether. Final separation and quantitation was achieved by a gas chromatograph in series with a Nermag R10-10 operated in the negative ion mode.

Serum TXB_2 was determined by radioimmunoassay following incubation of whole blood in a glass test tube at 37°C for 45 min (35).

Statistical analysis

The time to coronary occlusion was compared in the different treatment groups by the Kruskal-Wallis one-way analysis of variance (36). This is a nonparametric test and makes no assumptions as to the distribution of the data. Animals who failed to occlude were assigned a value equal to the maximum period of observation. However, this does not alter the statistical analysis that is based on ranks and not absolute values. The effects of treatments on serum TXB_2 and platelet aggregation were compared within groups by paired t test and between groups by one-way analysis of variance. Urinary metabolites were analyzed by two-way analysis of variance within groups. All data are expressed as the mean ± SEM.

Results

Serum TXB_2. U63,557a decreased serum TXB_2 by 86±3.9% ($n = 9$, $P < 0.001$) when given alone and by 93±1.7% ($n = 7$, $P < 0.001$) in combination with L636,499. The effects of the two treatments were not significantly different from each other. This reduction in serum TXB_2 persisted for at least 4 h in both groups. Pretreatment with aspirin exerted little further effect (96±1%, $n = 6$). In contrast, L636,499 induced only a small reduction in serum TXB_2 (23±8%, $n = 14$; $P < 0.05$), which by analysis of variance was not significantly different from vehicle alone (9±8%, $n = 8$).

Animal studies. In acute, open-chest experiments heart rate and blood pressure were unaltered by U63,577a and L636,499 given individually or combined. Coronary occlusion occurred in control animals in 74±6.7 min, similar to what we have reported previously (28). The time to coronary occlusion was prolonged 32±15% by U63,557a (Fig. 1) to 98±4.0 min ($n = 17$, NS). L636,499 had a more marked effect, prolonging the time to occlusion to 159±16 min ($n = 12$, $P < 0.01$). Indeed, three of the dogs failed to occlude over the 4-h observation period. In dogs treated with the combination of the two drugs coronary occlusion was prevented in five of nine experiments, the time to coronary occlusion being prolonged by 204±8% to 225±6.2 min ($P < 0.001$). Thus, combination of U63,557a and L636,499 was more effective in preventing and delaying coronary occlusion than either treatment alone ($P < 0.05$).

To further evaluate the effect of the combination therapy, we performed the same experiment in closed-chest animals where more prolonged observation was possible. Coronary oc-

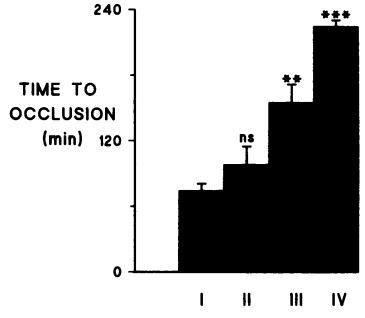

Figure 1. Time to coronary occlusion in acute experiments. Group I, vehicle. Group II, U63,557a. Group III, L636,499. Group IV, U63,557a + L636,499. (NS, not significant; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. control).

clusion occurred in 72±16 min (n = 8) in vehicle-treated animals, similar to acute experiments. As in the acute study, the combination of U63,557a and L636,499 was more effective than either treatment alone (Fig. 2), five of nine animals failing to occlude over 7 h of observation with a mean time to coronary occlusion of 341±39 min ($P < 0.001$ vs. controls, $P < 0.01$ vs. either treatment). Thus, U63,557a increased the time to coronary occlusion to a greater extent in the presence of the TXA_2/prostaglandin endoperoxide receptor antagonist, L636,499 (181±21% vs. 71±30%, $P < 0.01$), suggesting a synergistic interaction between these drugs. In contrast, the combination of U63,557a and L636,499 failed to alter the time to coronary occlusion in animals pretreated with aspirin (69±6 min, n = 5).

Platelet studies. Platelet aggregation to the TXA_2/prostaglandin endoperoxide analogue, U46619, and to arachidonic acid was unaltered in control experiments (Figs. 3 and 4). L636,499 inhibited the response to U46619 and arachidonic acid, increasing the threshold concentration of arachidonic acid for complete, irreversible aggregation from 0.23±0.02 to 0.77±0.09 mM (n = 16, $P < 0.001$). Combination of U63,557a and L636,499 exerted a more marked effect, completely abolishing the response to both U46619 and arachidonic acid.

In contrast, U63,557a alone had no effect on U46619-induced platelet aggregation. The effect of U63,557a on arachidonate-induced platelet aggregation was dependent on the concentration of the agonist. Thus, inhibition of platelet aggregation was paradoxically greater at higher concentrations of arachidonic acid with no inhibition demonstrated at 0.08 and 0.17 mM arachidonic acid (Fig. 4). This effect also occurred in vitro and was demonstrable with a structurally distinct TXA_2 synthase inhibitor, imidazo [1,5-2] pyridine-5-hexanoic acid (CGS13080) (Fig. 5). Furthermore, platelets failed to aggregate to ADP or U46619 after incubation of PRP with U63,557a 10 μg/ml and arachidonic acid 0.66 mM. This inhibition of platelet aggregation was prevented by pretreating the platelets with aspirin 10 μM for 10 min, suggesting that this reflected formation of a cyclooxygenase product.

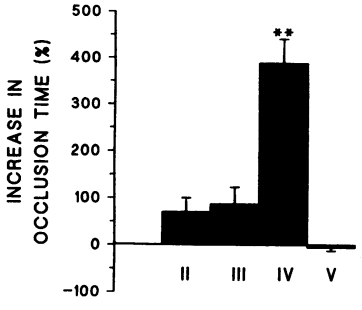

Figure 2. Percent increase in the time to coronary occlusion compared with vehicle-treated controls in chronic experiments. Group II, U63,557a. Group III, L636,499. Group IV, U63,557a + L636,499. Group V, aspirin + U63,557a + L636,499. ($^{**}P < 0.01$ vs. groups II, III, and V).

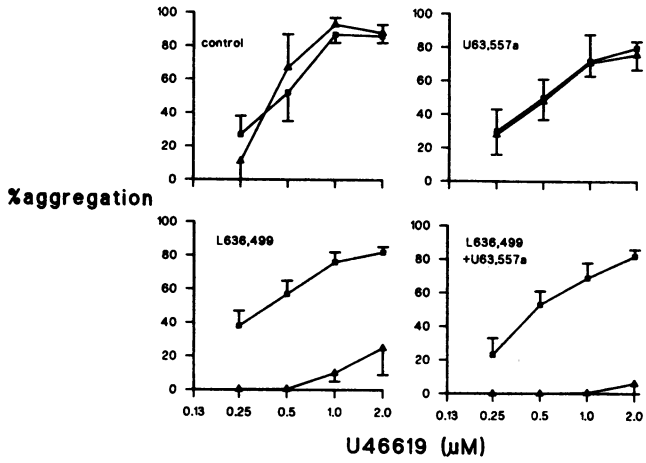

Figure 3. Ex vivo platelet aggregation to U46619. ■, predrug. ▲, postdrug.

Biochemical studies. Urinary excretion of 2,3-dinor-TXB_2 and 2,3-dinor-6-keto-$PGF_{1\alpha}$ was determined only in chronic experiments where artifactual production of the parent eicosanoids by surgical trauma is minimized (37). In all groups, urinary metabolite excretion was not significantly different on the day of study from presurgery levels. Urinary 2,3-dinor-TXB_2 increased significantly during induction of coronary thrombosis, from 871±118 to 1650±281 pg/mg creatinine ($P < 0.01$) and remained elevated after complete coronary occlusion (Fig. 6). U63,557a prevented the increase in 2,3-dinor-TXB_2 excretion when given alone and when combined with L636,499. This persisted over the period of observation, up to 7 h in some experiments. Similarly, pretreatment with aspirin prevented the increase in excretion of this metabolite. In contrast, 2,3-dinor-TXB_2 increased in dogs treated with L636,499 and continued to rise over the prolonged period of observation in these animals (Fig. 6).

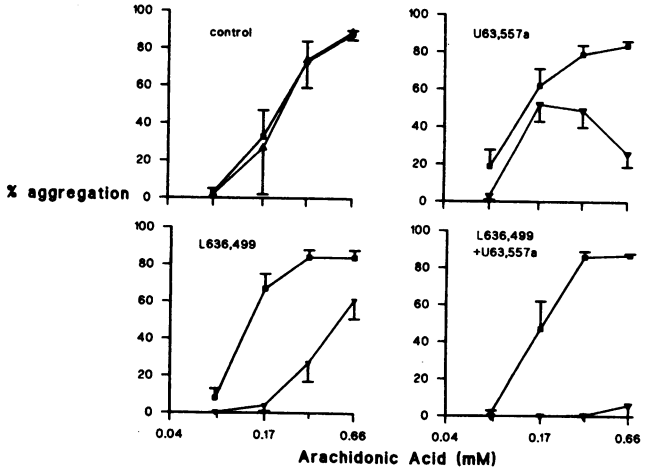

Figure 4. Ex vivo platelet aggregation response to arachidonic acid. ■, predrug, ▲, postdrug.

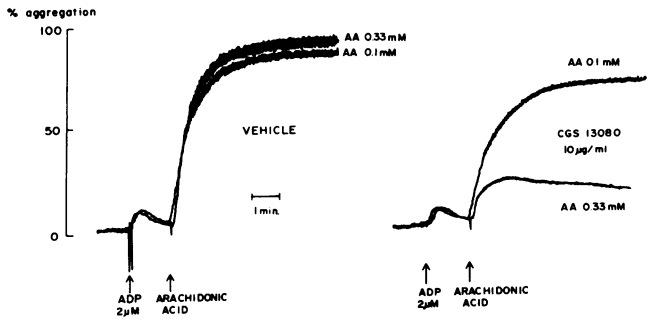

Figure 5. Platelet aggregation to arachidonic acid in canine PRP in the presence and absence of CGS13080 10 μg/ml. After incubation with the thromboxane synthase inhibitor platelet aggregation to 0.1 mM arachidonic acid is slightly inhibited. Increasing the concentration of arachidonic acid results in a more marked degree of platelet inhibition.

Urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ also increased both in control animals and in animals treated with L636,499 (Fig. 7), reaching a peak after complete coronary occlusion. This increase was more marked in dogs treated with U63,557a, particularly when combined with the TXA_2/prostaglandin endoperoxide receptor antagonist, L636,499, and persisted throughout the period of observation. Pretreatment with aspirin markedly blunted the increase in urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ seen with the combination therapy.

Discussion

These studies address the role of prostaglandin endoperoxides in modulating the response to TXA_2 synthase inhibitors in a chronic canine model of coronary thrombosis. This is a TXA_2 dependent model in that it is associated with increased thromboxane biosynthesis (37) and is inhibited by specific TXA_2/prostaglandin endoperoxide receptor antagonists (28). Biosynthesis of TXA_2 and PGI_2 was quantitated by excretion of their enzymatic metabolites in urine using highly specific assays. These methods avoid the artifacts inherent in the measurement of nonenzymatic, hydrolysis products (TXB_2 and 6-keto-$PGF_{1\alpha}$, respectively) and the trauma of blood sampling (38). Furthermore, eicosanoid biosynthesis was determined remote from the trauma of surgery, when TXA_2 and PGI_2 formation had returned to baseline. In open-chest experiments, excretion of the enzymatic metabolites of TXA_2 and PGI_2 is markedly increased, reflecting an increase in the formation of the parent eicosanoids by traumatized tissue and platelet activation at wound sites (37). However, by 5 d postsurgery, eicosanoid biosynthesis had fallen to preoperative levels, allowing detection of a thrombus-related increase in TXA_2 and PGI_2 biosynthesis that would have been largely obscured in open-chest experiments.

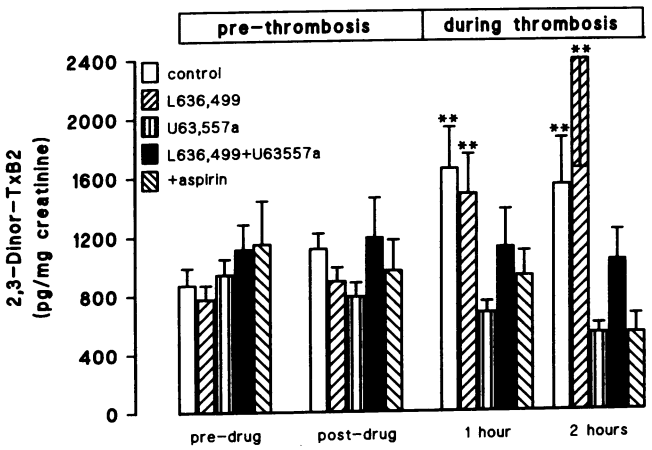

Figure 6. Urinary 2,3-dinor-TXB_2 before and after drug administration and during induction of coronary thrombosis in the chronic canine model. (**$P < 0.01$ vs. level before induction of coronary thrombosis.)

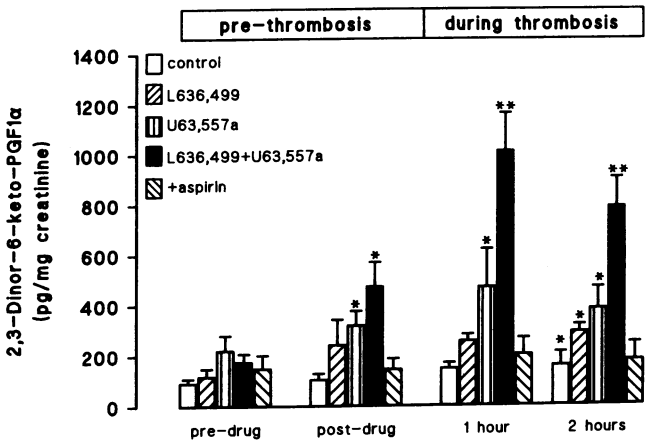

Figure 7. Urinary 2,3-dinor-6-keto-$PGF_{1\alpha}$ before and after drug administration and during induction of coronary thrombosis in the chronic canine model. (*$P < 0.05$; **$P < 0.01$ vs. level before induction of coronary thrombosis).

To examine the role of prostaglandin endoperoxides in the response to TXA_2 synthase inhibition in this model, we examined the interaction of L636,499 and U63,557a. L636,499 is a selective antagonist of the shared TXA_2/prostaglandin endoperoxide receptor (39, 40). Consistent with this, L636,499 did not inhibit TXA_2 or PGI_2 biosynthesis during coronary thrombosis in this model and prevented platelet aggregation to the endoperoxide analogue, U46619. The small reduction in serum TXB_2 following the administration of L636,499 has been noted previously for this and other TXA_2 antagonists (28, 41, 42) and may reflect inhibition of TXA_2-dependent platelet activation during incubation of the blood. U63,557a is a potent TXA_2 synthase inhibitor (43) and, in contrast to L636,499, markedly depressed serum TXB_2, prevented the increase in TXA_2 biosynthesis during coronary thrombosis and had no effect on U46619-induced platelet aggregation.

Despite their potent effects on thromboxane biosynthesis, TXA_2 synthase inhibitors exhibit limited platelet inhibitory activity both in vitro and ex vivo (7, 15–20). Similarly, in this study, U63,557a failed to inhibit coronary occlusion despite its effects on TXA_2 biosynthesis. Similar results have been reported in this model using a structurally distinct TXA_2 synthase inhibitor (19). Addition of the TXA_2/prostaglandin endoperoxide receptor antagonist, L636,499, enhanced the response to U63,557a without a further reduction in TXA_2 biosynthesis in vivo or in serum TXB_2 ex vivo. This suggests that the response to the TXA_2 synthase inhibitor was limited

by either incomplete inhibition of TXA_2 formation or by the accumulation of prostaglandin endoperoxides. Prostaglandin endoperoxides, precursors of TXA_2 in the cyclooxygenase pathway of arachidonic acid metabolism, activate a receptor shared with TXA_2 that induces platelet activation and vascular smooth muscle contraction (15, 44). In vitro studies demonstrate that prostaglandin endoperoxides can substitute for the proaggregatory effect of TXA_2 and overcome the effect of TXA_2 synthase inhibition on platelets (7, 15–17). In view of the marked inhibition of TXA_2 biosynthesis at doses of U63,557a used in this study, these data are consistent with the hypothesis that prostaglandin endoperoxides also limit the response to TXA_2 synthase inhibition in vivo.

This study also demonstrated that addition of the TXA_2 synthase inhibitor increased the response to the TXA_2/prostaglandin endoperoxide receptor antagonist. In part this may reflect a reduction in agonist formation. Alternatively, it may be due to increased biosynthesis of platelet inhibitory prostaglandins. In vitro studies have demonstrated that prostaglandin endoperoxides derived from platelets in the presence of a TXA_2 synthase inhibitor may be metabolized or shunted to platelet-inhibitory prostaglandins, including PGI_2 (22, 23) and PGD_2 (24, 25), by contiguous tissues and by the isomerase activity of plasma albumin, respectively. Indeed, this may be a major mechanism of their activity both in vitro (25) and in vivo (11). As evidence of a shunt to platelet inhibitory prostaglandins, there was a marked increase in PGI_2 formation, coincident with the inhibition of TXA_2 biosynthesis in animals treated with U63,557a alone or in combination with the TXA_2/prostaglandin endoperoxide receptor antagonist. This effect was most marked during induction of coronary thrombosis. Furthermore, the enhanced effect seen when U63,557a was combined with L636,499 was abolished by aspirin at a dose that prevented the increase in PGI_2 biosynthesis. These data, therefore, provide both functional and biochemical evidence of a shunt to platelet-inhibitory prostaglandins in the presence of a TXA_2 synthase inhibitor. Similar findings have recently been reported in human volunteers by Gresele and co-workers (26). In their studies, addition of a TXA_2 synthase inhibitor enhanced the effect of a TXA_2/prostaglandin endoperoxide receptor antagonist on bleeding time, an effect that was prevented by pretreatment with indomethacin.

The results of ex vivo platelet aggregation studies were also consistent with a role for platelet inhibitory prostaglandins in mediating the antiplatelet effects of U63,557a. Thus, inhibition of arachidonate-induced platelet aggregation by U63,557a was dependent on the concentration of arachidonic acid. At 0.08–0.17 mM arachidonic acid, U63,557a failed to inhibit platelet aggregation, consistent with the proaggregatory effects of prostaglandin endoperoxides. At higher concentrations of arachidonic acid, platelet activation was inhibited in the presence of U63,557a. This was also demonstrated with a second TXA_2 synthase inhibitor, CGS13080, suggesting that it was a specific effect of this class of compounds. After incubation of platelets with a high concentration of arachidonic acid and U63,557a, platelets failed to respond to ADP or U46619. This effect was not seen at lower concentrations of arachidonic acid and was prevented by pretreating the platelets with aspirin.

These findings suggest that platelet inhibition in this setting is due to formation of a cyclooxygenase product. Similar findings have been reported using human platelets. Indeed, inhibition of arachidonate-induced aggregation of human platelets by TXA_2 synthase inhibitors in vitro has been reported to largely reflect conversion of prostaglandin endoperoxides to PGD_2 by the isomerase activity of plasma proteins (24, 25). However, unlike canine platelets, human platelets do not demonstrate increased inhibition at higher concentrations of arachidonic acid (24, 25). PGD_2 inhibits canine platelets but only at concentrations one to two orders of magnitude greater than that required to inhibit human platelets (Fitzgerald, D. J., and G. A. FitzGerald, unpublished data). This may explain why inhibition of canine platelets is seen only at higher concentrations of arachidonic acid, where sufficient PGD_2 may be formed to overwhelm the proaggregatory effect of prostaglandin endoperoxides. Whether this mechanism is operative in vivo in the dog, therefore, will depend on the amount of substrate released at the site of platelet activation.

In conclusion, these studies demonstrate that prostaglandin endoperoxides modulate the response to TXA_2 synthase inhibition in a chronic, close-chest model of coronary thrombosis. First, endoperoxides may limit the response by substituting for the proaggregatory effect of TXA_2. Second, they may be metabolized to platelet-inhibitory prostaglandins. The functional significance of the shunting of prostaglandin endoperoxides to platelet inhibitory products can be demonstrated when their proaggregatory effect is prevented by an antagonist of the shared TXA_2/prostaglandin endoperoxide receptor. The resulting synergistic interaction between a TXA_2 synthase inhibitor and a TXA_2/prostaglandin endoperoxide receptor antagonist renders this combination a very effective antiplatelet regimen of potential therapeutic importance.

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