

Aurintricarboxylic Acid in a Canine Model of Coronary Artery Thrombosis

John Strony, MD, Martin Phillips, MD, David Brands, BS,
Joel Moake, MD, and Burt Adelman, MD

Platelet thrombus formation occurs at sites of severe arterial narrowing where shear stress is elevated. Shear stress appears to induce platelet aggregation in vitro by means of initiation of von Willebrand factor binding to platelet glycoprotein Ib. Recent in vitro studies have demonstrated that aurintricarboxylic acid can inhibit shear stress-induced platelet aggregation. This effect is mediated by aurintricarboxylic acid binding to von Willebrand factor; this binding results in inhibition of von Willebrand factor interaction with glycoprotein Ib. In this study, we examined the effect of aurintricarboxylic acid on platelet-dependent cyclic flow reductions (CFRs) in a canine coronary stenosis model. In dose-response experiments, six animals received 4 mg/kg aurintricarboxylic acid by bolus infusion, followed by 1 mg/kg every 10 minutes. Total inhibition of CFRs was observed in all animals after 6.7 mg/kg aurintricarboxylic acid; CFRs could not be reinitiated by the thromboxane A₂ analogue U46619. Continuous infusion of epinephrine (0.4 µg/kg/min) caused CFRs to return; however, 3.7 mg/kg additional aurintricarboxylic acid again induced total inhibition of CFRs. In addition, five animals received a bolus infusion of 10 mg/kg aurintricarboxylic acid, which caused total inhibition of CFRs. The average area of stenosis in the constricted vessels was 83%, and shear stress at the site of constriction averaged 350 dynes/cm². Aurintricarboxylic acid did not alter hemodynamics, thrombin time, platelet count, or ADP/epinephrine-induced platelet aggregation. These data indicate that platelet glycoprotein Ib-von Willebrand factor interactions are important during coronary occlusion and that aurintricarboxylic acid can inhibit coronary thrombosis associated with coronary constriction. (*Circulation* 1990;81:1106-1114)

Platelets are an important component of occlusive thrombi that form in arteries diseased by atherosclerosis.¹ Platelet activation in atherosclerotic vessels is probably initiated by a number of different processes, including atherosclerotic plaque rupture and shear stress. Plaque rupture exposes thrombogenic subendothelial matrix components that bind von Willebrand factor and cause platelet adherence and aggregation. In addition, thrombin generation initiated by plaque disruption further contributes to platelet aggregation.

Platelet-dependent thrombus formation may also be induced by high shear stress occurring at arterial

sites where underlying atheroma or hemorrhage have caused severe arterial narrowing. Phillips, Moake, and colleagues²⁻⁴ recently demonstrated that shear stress-induced platelet aggregation is dependent on large von Willebrand factor multimers and the platelet surface receptors, glycoproteins Ib and IIb/IIIa. In addition, they postulated that localized endothelial cell dysfunction can result in release of unusually large von Willebrand factor multimers that accelerate this process.^{2,3} Weiss et al⁵ have also reported that von Willebrand factor interactions with both glycoproteins Ib and IIb/IIIa are essential for platelet aggregation to occur under conditions of increased shear stress. The pivotal role for von Willebrand factor in shear stress-induced platelet aggregation is further supported by the observations that neither fibrinogen nor arachidonate metabolism contributes to shear-induced platelet aggregation and that von Willebrand factor binding to glycoprotein Ib can result in platelet activation.⁴⁻⁷ Thus, it is possible that shear stress-induced von Willebrand factor-dependent platelet aggregation is a cause of platelet thrombus formation in constricted arteries.

We have previously reported² that aurintricarboxylic acid, a triphenylmethyl dye compound, inhibits

From the Hunter Holmes McGuire Veterans Administration Medical Center, Medical College of Virginia, Richmond, Virginia, and The Baylor College of Medicine, The Methodist Hospital, and Rice University, Houston, Texas.

Supported by The Council for Tobacco Research, USA, Inc., the Veterans Administration Research Program, the American Heart Association, Virginia Affiliate, the A.D. Williams Foundation, and National Institutes of Health grant HL-35387. M.P. is the recipient of an American Heart Association Clinician-Scientist Award.

Address for reprints: Burt Adelman, MD, IIK, VA Medical Center, 1201 Broad Rock Blvd., Richmond, VA 23249.

Received August 7, 1989; revision accepted November 28, 1989.

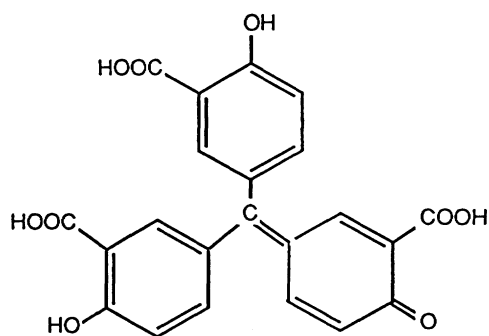


FIGURE 1. Chemical structure of aurintricarboxylic acid.

platelet aggregation in a shear field. Our data indicated that aurintricarboxylic acid bound to von Willebrand factor and inhibited von Willebrand factor interaction with glycoprotein Ib. Aurintricarboxylic acid had no effect on platelet metabolic processes and did not inhibit platelet aggregation induced by ADP or thrombin. In the present study, we have evaluated the ability of aurintricarboxylic acid to inhibit platelet-dependent thrombus formation in an *in vivo* model of coronary thrombosis. In this model system, described by Folts and colleagues,^{8,9} cyclic reductions in coronary blood flow occur due to platelet-dependent thrombi forming at the site of a coronary stenosis created by the placement of a fixed constrictor.

Methods

Materials

Conditioned dogs of both sexes weighing 20–25 kg were used in this study. The trisodium salt of aurintricarboxylic acid (Figure 1) was obtained from Aldrich Chemical, Milwaukee, Wisconsin. Before administration, it was dissolved in phosphate-buffered saline, pH 7.4, and sterilized by filtration through a 22- μ m filter. ADP, epinephrine, and trisodium citrate were obtained from Sigma Chemical, St. Louis, Missouri. The thromboxane A₂ analogue U46619 was obtained from Cayman Chemical, Ann Arbor, Michigan. Epinephrine for infusion was purchased from Abbott Laboratories, North Chicago, Illinois. All other reagents were reagent grade or better and were obtained from standard commercial suppliers.

Surgical Preparation

In this study, we used the canine acute coronary occlusion model developed by Folts and colleagues.^{8,9} Mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg *i.v.*), intubated with a cuffed endotracheal tube, and ventilated on room air by a respirator (Harvard, Apparatus, South Natick, Massachusetts). Body temperature was maintained at 37–39° C with radiant heat and a heating pad. An 8F arterial sheath and venous catheter were inserted into the right femoral artery and vein, respectively, for continuous monitoring of blood pressure, administration of fluids, and subsequent angiography. All fluids infused were warmed to body temperature. A left thoracotomy was performed at the fifth intercos-

tal space, and the heart was exposed. A small pericardial window was created at the level of the mid-left circumflex coronary artery, and a 2-cm segment of the left circumflex artery was exposed and dissected free from the surrounding tissue. All branches of the circumflex artery within the dissected segment were ligated. A pulsed-wave Doppler flow probe (Biomedical Engineering, University of Iowa, Iowa City) was placed around the proximal portion of the dissected vessel, followed by a calibrated electromagnetic flow probe (model 501B, Carolina Medical Equipment). A small polyethylene catheter was then placed into the left atrium and connected to a pressure transducer (Gould, Cleveland, Ohio) for monitoring of left atrial pressures and for later administration of the thromboxane A₂ mimetic, U46619.

After the animal was allowed to stabilize for approximately 15 minutes, baseline coronary blood flow, blood pressure, heart rate, and left atrial pressures were recorded and displayed on an eight-channel recorder (model 2800, Gould). To provide control values for subsequent comparison, the coronary artery was occluded for 20 seconds with suture and then released with recording of the hyperemic response. After coronary blood flow returned to normal, a cylindrical plastic (Lexan) constrictor modeled after those described by Folts and colleagues^{8,9} was placed on the isolated segment of the circumflex artery 1 cm distal to the Doppler and electromagnetic flow probes. In each animal, we used a constrictor that attenuated or abolished the hyperemic response after coronary occlusion but that did not cause an immediate reduction in blood flow. Cyclic flow reductions (CFRs) due to platelet-dependent thrombus formation occurred shortly after placement of the constrictor. These gradual declines in coronary blood flow velocity were occasionally interrupted by spontaneous restorations in flow through the stenotic artery. In most instances, blood flow through the constricted coronary artery was restored by manually moving the constrictor around the artery after the flow signal declined to near zero. Once CFRs developed, the animals were continuously monitored for 30 minutes so that baseline CFR frequency, phasic and mean coronary blood flow, blood flow velocity, heart rate, and left atrial and systemic blood pressure responses could be documented.

In Vivo Response to Aurintricarboxylic Acid

Animals instrumented to produce CFRs were given aurintricarboxylic acid or placebo after a 30-minute baseline monitoring period. The first group of animals received incremental doses of aurintricarboxylic acid. After an initial dose of 4 mg/kg, additional 1 mg/kg doses were given every 10 minutes until cyclic flow reductions were totally inhibited. The second group of animals received a single bolus dose of aurintricarboxylic acid equivalent to the average inhibitory dosage determined from the incre-

mental dose study. The third group of animals received the drug vehicle alone.

The effect of aurintricarboxylic acid or drug vehicle on hemodynamic response and cyclic flow reductions was continuously monitored in each animal. In addition, the following parameters were also measured before, during, and after drug infusion: platelet count, thrombin time, and platelet aggregation in response to ADP/epinephrine.

The aurintricarboxylic acid used in this study comprises monomers and polymers of aurintricarboxylic acid that possess the in vitro capacity to inhibit von Willebrand factor-platelet glycoprotein Ib interactions. It also contains some inactive aurintricarboxylic acid species (M. Weinstein, M. Phillips, and J. Moake, unpublished data). To ensure uniform effect and comparability from animal to animal, all experiments were conducted with aurintricarboxylic acid obtained from a single product lot (4513LL, Aldrich Chemical).

Once cyclic flow reductions were completely abolished, 6 μg U46619 was applied topically over the constricted vessel. This was repeated by a second 6- μg infusion into the left atrium. If no recurrence of CFRs was noted, a 0.4 $\mu\text{g}/\text{kg}/\text{min}$ epinephrine infusion was initiated. On the return of CFRs, 1.0 mg/kg i.v. bolus doses of aurintricarboxylic acid were administered every 10 minutes until CFRs were again totally abolished.

Drug effect on CFRs was analyzed in two ways. First, we determined the effect of drug administration on the average frequency of CFRs normalized to CFRs per hour. Second, we measured the effect of drug on the average rate of change of coronary blood flow (slope) during each CFR (cm^3/min).

Calculation of Shear Stress

Shear stress at the point of maximal stenosis was calculated. After completion of the drug intervention study, each animal underwent coronary angiography using Iohexol, a nonionic contrast agent (Winthrop Pharmaceuticals, New York, New York). Injection took place at the point of peak blood flow if CFRs were present. Direct digital images were acquired as well as 35-mm cinefilm and high-resolution videotape at orthogonal angles. The vessel angiographs were analyzed by an image processing unit (model 2400, Adac Laboratories, California) for determination of length of stenosis, luminal diameter of the normal and stenosed segments, and area and tapering of the stenosed region. The quantitative angiographic methods used have been previously validated by Mancini et al.¹⁰

Shear stress at the site of constriction was calculated using a laminar boundary layer equation derived for use in coronary arteries by Back et al.¹¹ and Lipowsky et al.¹² This formula calculates shear stress at the point of maximal stenosis during peak diastolic coronary blood flow.

Coagulation Studies

The in vitro and ex vivo effect of aurintricarboxylic acid on platelet aggregation was evaluated. In vitro studies were conducted on dog platelet-rich plasma prepared from blood drawn by a double-syringe technique into trisodium citrate anticoagulant (final anticoagulant concentration, 0.38%). The dogs used for this purpose had been trained to undergo venipuncture and phlebotomy. Aurintricarboxylic acid dissolved in phosphate buffer was added to platelet-rich plasma to achieve final concentrations ranging from 0 to 600 $\mu\text{mol}/\text{l}$. Buffer alone was added to control samples. Platelet aggregation after addition of both 12.5 $\mu\text{mol}/\text{l}$ ADP and 0.25 $\mu\text{mol}/\text{l}$ epinephrine was measured turbidimetrically with an aggregometer (Sienco, Morrison, Colorado).

Ex vivo analysis of ADP/epinephrine-induced platelet aggregation was performed on platelet-rich plasma obtained from study and control animals before and after administration of aurintricarboxylic acid or buffer. The platelet aggregation response after drug infusion was compared with pretreatment response. Thrombin time determinations on plasma were performed by the method of Clauss¹³ in a fibrometer (BBL Microbiology Systems, Cockeysville, Maryland).

Effect of Aurintricarboxylic Acid on Shear-Induced Platelet Aggregation In Vitro

Shear-induced platelet aggregation was studied as previously described by using the Ferranti cone and plate viscometer (Ferranti Electric, Commack, New York).^{2,3} Samples of canine platelet-rich plasma were applied to the plate in the presence or absence of aurintricarboxylic acid or buffer. Before and after shearing, samples were taken for particle counting. A shear force of 180 dynes/cm^2 was applied for 30 seconds. Particle counts were performed in an electronic particle counter and channelizer (model ZBI, Coulter Diagnostics, Hialeah, Florida). The sample volume for counting was 100 μl , and the aperture setting was 50 μm . Particles with sizes $\pm 20\%$ of the mean platelet distribution in the unsheared samples were considered as single platelets. The disappearance of single platelets could be accounted for by the formation of platelet aggregates. Thus, the percent decrease in single platelets was directly related to the percent increase in platelet aggregates. All counts were done in duplicate.

Statistical Analysis

All values are expressed as mean \pm SEM. The data were analyzed by ANOVA for repeated measures. The calculated p values were adjusted to account for multiple data points derived from each animal. Paired Student's t test was used to determine whether a significant change in a variable occurred within the animals studied. A value of $p < 0.05$ was used to define a significant difference between values.

Results

Baseline Hemodynamics and Constriction-Induced Changes in Coronary Artery Blood Flow

Of the 16 dogs studied, 15 dogs exhibited CFRs after placement of the coronary artery constrictor. Coronary blood flow in the constricted circumflex artery was minimally reduced; however, maximal hyperemic response after a 20-second occlusion declined from $332 \pm 38\%$ of basal flow in nonoccluded vessels to $143 \pm 14\%$ of basal flow in constricted vessels. These parameters were comparable in all experimental and control groups. Heart rate and blood pressure remained unchanged for the duration of the study.

Effect of Aurintricarboxylic Acid on Cyclic Flow Reductions

After CFRs were monitored for 30 minutes, aurintricarboxylic acid was administered to each of six animals in incremental doses of 1 mg/kg every 10 minutes after an initial 4 mg/kg bolus dose. Before drug infusion, CFR frequency was 20 ± 2 cycles/hr, and the mean decrease in blood flow was 10 ± 3 cm³/min. In each animal, aurintricarboxylic acid abolished CFRs in a dose-related fashion. Figure 2 illustrates the typical response to aurintricarboxylic acid infusion during the occurrence of CFRs. Figure 3 summarizes the data from all six animals and illustrates the dose-response relation between the cumulative amount of aurintricarboxylic acid infused and its effect on both the frequency of CFRs and their course (decrease in blood flow over time). The average dose of aurintricarboxylic acid needed for total inhibition of CFRs was 6.7 ± 0.4 mg/kg.

In three animals, after complete inhibition of CFRs was achieved, 6 μ g of the thromboxane A₂ mimetic U46619 was applied directly to the vessel at the site of constriction and also injected as a bolus dose through the left atrial catheter. Although a modest increase in heart rate and blood pressure was observed, CFRs did not return. In three other animals, also after inhibition of CFRs, epinephrine was administered as a continuous infusion at a rate of 0.4 μ g/kg/min. Epinephrine caused an increase in blood pressure, heart rate, and coronary blood flow lasting approximately 10 minutes. In all three animals, CFRs returned within 6 minutes of initiating the epinephrine infusion. While the infusion was continued, additional bolus doses of aurintricarboxylic acid were administered every 10 minutes. After infusion of an additional 3.7 ± 0.7 mg/kg, total inhibition of CFRs was again achieved (Figure 2).

From the studies described above, we determined that a total dose of 10 ± 0.6 mg/kg aurintricarboxylic acid should induce total inhibition of cyclic flow reductions, even in the presence of epinephrine. To evaluate this, four animals were given 10 mg/kg single-bolus infusions. We observed total inhibition of CFRs lasting 173 ± 8 minutes; the onset of effect was seen within 1–3 minutes of drug administration. There were no hemodynamic changes associated with

the effect of aurintricarboxylic acid. Mean heart rate and blood pressure remained stable throughout the course of each experiment (Table 1). Similarly, in another animal, a single 10 mg/kg bolus infusion of aurintricarboxylic acid inhibited CFRs occurring during constant infusion of epinephrine (0.4 μ g/kg/min).

Four animals served as controls. After initiation of CFRs and continuous monitoring for 30 minutes, each animal was given a bolus infusion of the phosphate buffer used as the drug vehicle. Over a 3-hour period after buffer infusion, the frequency of CFRs did not change significantly (Table 1).

Calculated Shear Stress in Constricted Vessels

Quantitative coronary angiographic analysis of the stenosed coronary artery was performed in all animals receiving aurintricarboxylic acid. The percent vessel stenosis averaged 83%, with a minimal cross-sectional area of 0.94 mm². Mean calculated shear stress at peak diastolic flow was 403 ± 114 dynes/cm² in animals that received incremental doses of aurintricarboxylic acid and 299 ± 117 dynes/cm² in animals that received a bolus of aurintricarboxylic acid (the difference in shear stress between these groups was not significant, $p > 0.5$) (Table 2).

Analysis of the dose-response experiments did not indicate any correlation between the degree of shear stress at the site of constriction and total amount of aurintricarboxylic acid needed to inhibit CFRs. Moreover, there was no correlation noted between the inhibitory dose of aurintricarboxylic acid and the percent vessel lumen stenosis, minimal cross-sectional area of the stenosed vessel, or peak coronary blood flow. CFRs returned during intravenous epinephrine challenge in all animals studied. However, there was no appreciable augmentation of shear stress during the epinephrine infusion period.

Effect of Aurintricarboxylic Acid on Platelet and Clotting Parameters

Platelet aggregation studies were performed on canine platelet-rich plasma to which aurintricarboxylic acid was added in vitro and on platelet-rich plasma prepared from animals that were receiving aurintricarboxylic acid. No inhibition of ADP/epinephrine-induced platelet aggregation was observed when 100–600 μ mol/l aurintricarboxylic acid was added to normal platelet-rich plasma ex vivo. Similarly, thrombin time determinations were unaffected by the addition of 10–400 μ mol/l aurintricarboxylic acid to canine normal plasma.

Serial blood samples were obtained from 14 dogs constituting all three groups (dose-response, single dose, and control). Thrombin time, platelet count, and ADP/epinephrine-induced platelet aggregation studies were performed (Table 3). Baseline platelet count, obtained before initiation of CFRs, averaged $357,000 \pm 37,000/\mu$ l with no significant difference noted among the three animal groups. After CFRs had occurred for approximately 2 hours, the platelet count in the control animals decreased by an average

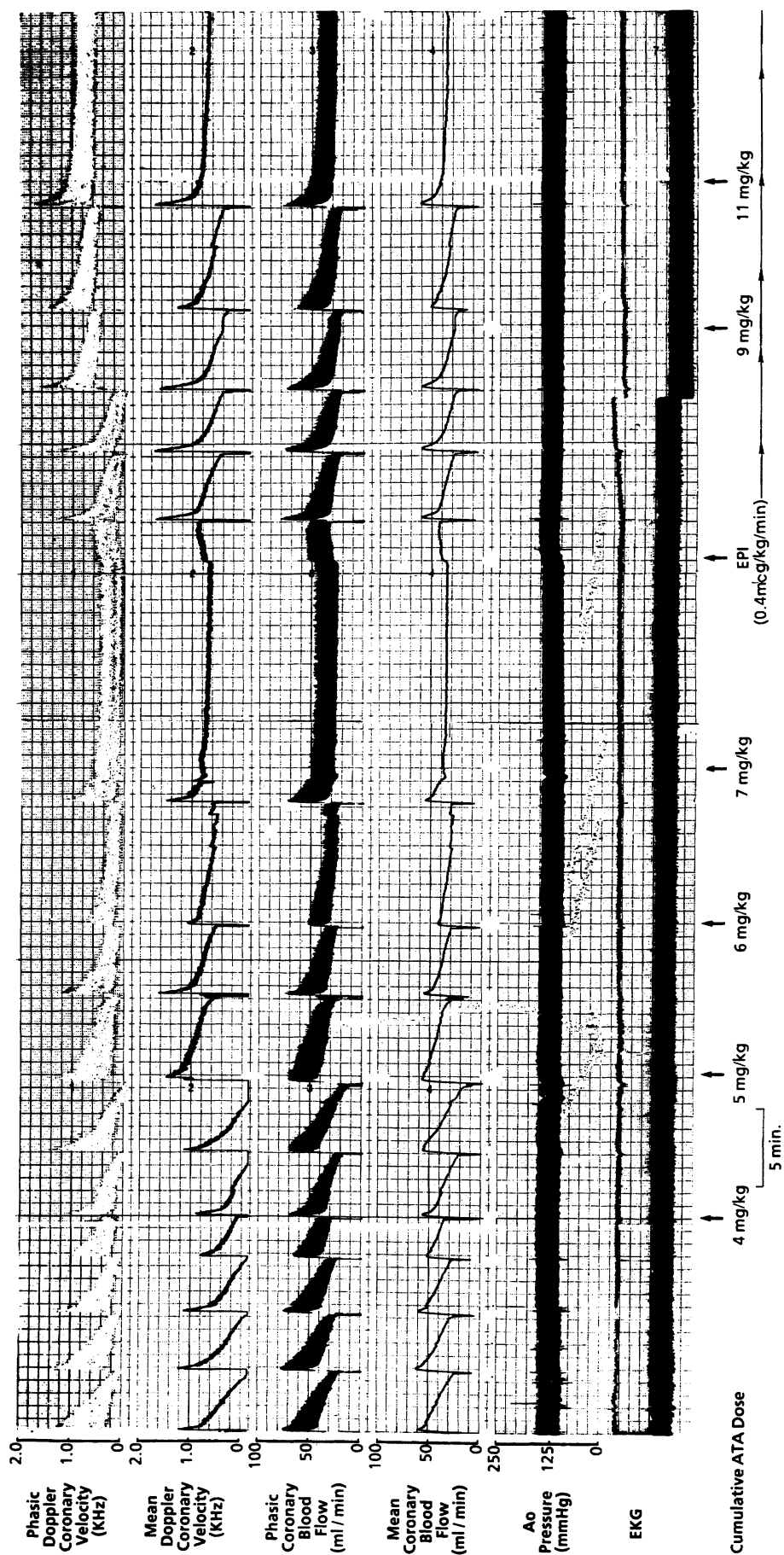


FIGURE 2. Actual physiologic recordings from an experiment showing the effect of aortic atherosclerotic acid (ATA) on cyclic flow reductions. The tracings indicate phasic and mean Doppler coronary velocity, phasic and mean coronary blood flow, phasic aortic (Ao) pressure, and electrocardiogram (ECG) during the occurrence of cyclic flow reductions. At the indicated time point, the animal received a bolus infusion of 4 mg/kg ATA followed by 1 mg/kg additional doses. Cyclic flow reductions were totally inhibited after a cumulative dose of 7 mg/kg ATA. After an approximate 15-minute period, a continuous infusion of 0.4 μ g/kg/min epinephrine (EPI) was begun. A modest rise in blood pressure and heart rate occurred that was followed by return of cyclic flow reductions. Cyclic flow reductions were again abolished by infusion of additional doses of ATA.

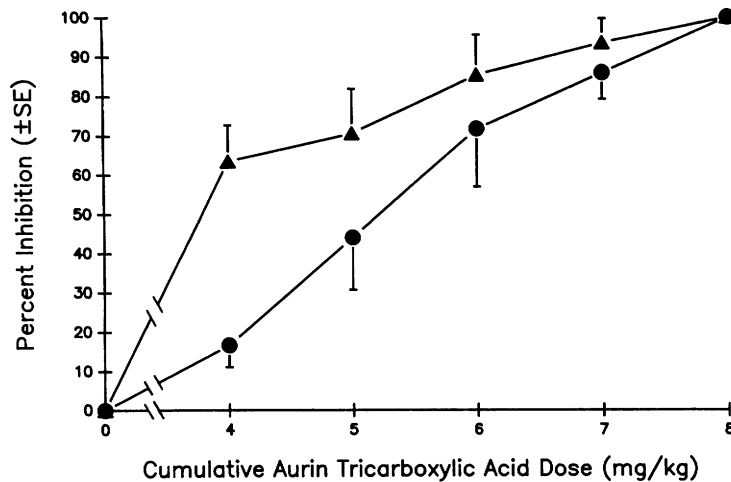


FIGURE 3. Plot of aurintricarboxylic acid effect on cyclic flow reductions after intermittent bolus infusion. After initial infusion of 4 mg/kg aurintricarboxylic acid, each animal received a 1 mg/kg dose every 10 minutes until cyclic flow reductions were totally inhibited. ANOVA indicated that cumulative doses of 6 mg/kg or greater caused significant reduction in cyclic flow reduction frequency and slope ($p < 0.001$). ●—●, Cyclic flow reduction frequency; ▲—▲, decrease in the rate of decline of blood flow (slope=ml/min) during each cycle; $n=6$.

15±11%. In those animals given aurintricarboxylic acid, platelet counts remained stable with only a 2±10% decrease noted. This difference in platelet count decrease between the control and aurintricarboxylic acid-treated animals was not statistically significant.

Effect of Aurintricarboxylic Acid on In Vitro Shear Stress-Induced Platelet Aggregation

To confirm the ability of aurintricarboxylic acid to inhibit shear-induced aggregation of canine platelets, we conducted a series of in vitro studies similar to those reported with human platelets.² In vitro canine platelets in platelet-rich plasma demonstrated consistent shear stress-induced platelet aggregation. The extent of shear stress-induced aggregation was determined by calculating the percent decline in single platelets after

shear. When a shear stress of 180 dynes/cm² was applied for 30 seconds to canine platelet-rich plasma, the platelet count declined 55±28% (mean±SD) of preshear control values. The addition of aurintricarboxylic acid to platelet-rich plasma inhibited shear stress-induced aggregation. At a concentration of 350 μmol/l, aurintricarboxylic acid totally inhibited shear stress-induced aggregation (final platelet count 108±20% of preshear platelet count). This is in marked contrast to the lack of inhibition by aurintricarboxylic acid of ADP/epinephrine-induced aggregation in the aggregometer.

We also examined the effect of aurintricarboxylic acid on shear stress-induced aggregation of washed canine platelets suspended in buffer containing very large human von Willebrand factor multimers. These von Willebrand factor species are obtained by adding supernatant from cultured human umbil-

TABLE 1. Hemodynamic Parameters in Animals With Cyclic Flow Reductions Treated With Aurintricarboxylic Acid

Group	HR (beats/min)	AoM (mm Hg)	CFR (CFRs/hr)	Slope (ml/min)	Phasic flow (ml/min)
Control ($n=4$)					
Baseline	143±13	122±6
Constricted	157±8	124±7	15±3	11.0±0.2	74±10
1 hour	157±7	112±4	16±2	9.3±0.1	78±11
2 hour	150±12	106±4	16±2	7.2±1.3	67±6
3 hour	151±15	98±7	17±2	8.5±1.1	72±8
Incremental dose ($n=6$)					
Baseline	123±11	127±8
Constricted	105±18	122±5	20±2	10.0±3.3	56±8
After ATA	120±12	121±4	0	0	62±10
Bolus dose ($n=4$)					
Baseline	111±12	129±6
Constricted	103±10	127±8	18±6	7.4±0	44±3
After ATA	115±8	128±13	0	0	43±10

Values are expressed as mean±SEM.

HR, heart rate; AoM, mean aortic pressure; CFR, cyclic flow reduction; slope, rate of decrease in blood flow during each CFR; phasic flow, phasic or peak diastolic coronary blood flow; ATA, aurintricarboxylic acid.

Control animals received drug vehicle only. Incremental-dose animals received an average of 6.71±0.42 mg/kg ATA. Bolus-dose animals received 10 mg/kg ATA.

TABLE 2. Analysis of the Constricted Coronary Artery Segment and Shear Stress in Animals Treated With Aurintricarboxylic Acid

	Normal vessel diameter (mm)	Stenosis (%)	MCA (mm ²)	Shear stress (dynes/cm ²)
Incremental ATA	5.9±0.8	82.1±4.3	0.88±0.14	403±114
Bolus ATA	5.6±1.1	84.4±2.6	1.01±0.26	299±117*

Values are mean±SEM.

MCA, minimal cross-sectional area; ATA, aurintricarboxylic acid.

*Differences between groups are not significant ($p>0.5$).

ical vein endothelial cells to the platelet suspension.^{2,4} Just as with platelet-rich plasma, shear stress aggregated these washed platelets, and the response was blocked by 350 μ mol/l aurintricarboxylic acid. Canine platelets suspended in buffer alone did not aggregate in the shear field.

Discussion

The studies presented in this report demonstrated the in vivo efficacy of aurintricarboxylic acid in preventing platelet-dependent thrombus formation in the experimental model of coronary stenosis developed by Folts and colleagues.^{8,9} In dose-response experiments, total inhibition of CFRs occurred after cumulative administration of 6.7 mg/kg aurintricarboxylic acid. Epinephrine caused a return of CFRs that were inhibited by infusion of an additional 3.7 mg/kg aurintricarboxylic acid. In single-dose infusion studies, 10 mg/kg aurintricarboxylic acid abolished CFRs within 3 minutes of injection, and inhibition lasted for approximately 175 minutes. No adverse hemodynamic or coagulation effects were noted during the course of these experiments.

Although Folts and colleagues^{9,14,15} and Eidt et al¹⁶ have demonstrated that CFRs result from platelet thrombus formation within a constricted segment of a coronary artery, the actual mechanism by which platelets are activated at the site of stenosis is unresolved. With the same model system used in our study, various investigators^{15,17-24} have demonstrated that thrombus formation can be prevented by meta-

bolic inhibition of platelet activation, by blockade of the glycoprotein IIb/IIIa receptor, or by infusion of antibody that binds to von Willebrand factor and inhibits von Willebrand factor-glycoprotein Ib binding. Thus, data indicate that both platelet activation and the platelet surface glycoproteins Ib and IIb/IIIa are necessary for thrombus formation within constricted coronary arteries. However, these studies did not identify the specific process that initiated platelet activation.

We suspect that shear stress may be an important cause of platelet activation and aggregation in constricted coronary arteries. Other investigators²⁻⁴ have already suggested this, and recent data from in vitro test systems confirmed that shear stress can induce platelet aggregation. In this study, we found that shear stress was elevated at the site of coronary constriction and that the average value obtained (350 dynes/cm²) was greater than the shear stress needed to aggregate canine platelets in vitro (180 dynes/cm²).

Phillips et al² demonstrated that large von Willebrand factor multimers were necessary for shear stress-induced platelet aggregation. Platelet activation in a shear stress field occurred in the absence of thrombin generation and did not require platelet secretion or exogenous fibrinogen. It is possible that shear stress initiates von Willebrand factor binding to glycoprotein Ib, which results in platelet activation. This mechanism would be consistent with the observation that platelet activation occurred after spontaneous binding of asialo-von Willebrand factor to glycoprotein Ib.^{6,7}

Recently Phillips et al² reported that aurintricarboxylic acid inhibited shear stress-induced platelet aggregation in vitro. Their data indicated that this effect was mediated by means of aurintricarboxylic acid binding to von Willebrand factor, which prevented its interaction with glycoprotein Ib. Based on these data and those discussed above, it seemed appropriate to test the effect of aurintricarboxylic acid on platelet thrombus formation in vivo. Our data clearly indicated that aurintricarboxylic acid was an effective inhibitor of platelet thrombus formation at sites of arterial constriction and elevated shear stress.

Shear stress was elevated at the site of constriction in all animals studied; however, there did not appear to be a direct correlation between the magnitude of shear stress achieved and the total dose of aurintricarboxylic acid needed to inhibit CFRs. It is possible that the dosage needed correlated more closely with plasma von Willebrand factor levels. This would be consistent with the previous in vitro finding² that the degree of inhibition of shear stress-induced platelet aggregation by aurintricarboxylic acid was directly dependent on its concentration and that of von Willebrand factor in the reaction mixture.

To validate our in vivo observations, we examined the effect of aurintricarboxylic acid in vitro in canine platelet-rich plasma. As observed with human platelets, shear stress caused rapid aggregation of canine platelets. This response was totally inhibited by addi-

TABLE 3. Coagulation Parameters in Animals Receiving Aurintricarboxylic Acid

	Platelet count (platelets/ μ l)	Inhibition of aggregation (%)	Change in thrombin time (%)
Control animals (n=4)			
Baseline	373,000±26,000	0	...
1 hour	321,000±54,000	2.7±11.6	0
ATA-infused animals (n=10)			
Baseline	357,000±37,000	0	...
After ATA	351,000±28,000	0	0

Values are mean±SEM.

ATA, aurintricarboxylic acid.

Baseline values are those obtained 30 minutes after initiation of cyclic flow reductions. Aggregation and thrombin time values at baseline and later are compared with those obtained before initiation of cyclic flow reductions.

tion of aurintricarboxylic acid to the platelet-rich plasma. In contrast, aurintricarboxylic acid added in vitro to canine platelet-rich plasma had no inhibitory effect on ADP/epinephrine-induced platelet aggregation. Similarly, the aggregation response of platelets obtained from animals receiving enough aurintricarboxylic acid to totally inhibit CFRs appeared normal.

It remains possible that the mechanism by which aurintricarboxylic acid inhibited platelet thrombus formation in vivo was more complex than that implied by in vitro studies.² We observed that epinephrine infusion caused CFRs to return after initial inhibition by aurintricarboxylic acid. It is not known how epinephrine infusion reinitiates CFRs; however, we did not detect a significant increase in shear stress associated with its infusion. Thus, further studies seem warranted to further characterize the platelet inhibitory action of aurintricarboxylic acid in vivo.

Coagulation studies did not identify an adverse effect of aurintricarboxylic acid. Platelet counts did not decline significantly in the control or experimental treatment groups. Fibrinogen concentration and coagulability were also unaffected by aurintricarboxylic acid. Thus, abolition of CFRs did not result from platelet destruction or inhibition of fluid phase coagulation. No other acute toxic effects of aurintricarboxylic acid were detected. In particular, we did not observe any effect on blood pressure or heart rate. Currently, little data is available about possible acute or chronic adverse effects of aurintricarboxylic acid; therefore, its potential use as a pharmaceutical agent is entirely unknown.

Direct inhibition of platelet interaction with adhesive glycoproteins appears to be an effective means of preventing acute arterial occlusion in experimental models of coronary stenosis. Recent studies by our laboratory¹⁹ and others²⁰ have demonstrated that blockade of the Agr-Gly-Asp sequence-receptor region of the glycoprotein IIb/IIIa complex inhibits CFRs in stenosed canine coronary arteries. The effect of aurintricarboxylic acid is unique: it inhibits glycoprotein Ib-mediated platelet adhesion and subsequent aggregation. This effect is von Willebrand factor dependent, fibrinogen independent, and effective in high shear fields.²⁻⁵

The results of this study using the model of coronary thrombus formation described by Folts and colleagues^{8,9} indicated that aurintricarboxylic acid was a potent inhibitor of platelet-dependent thrombus formation. Our observations suggest that inhibition of glycoprotein Ib-von Willebrand factor interaction may prove to be an effective means of preventing acute arterial thrombosis. How closely these studies relate to events that occur in human disease cannot be said with certainty; however, aurintricarboxylic acid holds promise as a prototype for a new class of arterial antithrombotic agents.

References

- DeWood MA, Spores J, Notske R, Mouser LT, Burroughs R, Golden MS, Lang HT: Prevalence of total coronary occlusion during the early hours of transmural myocardial infarction. *N Engl J Med* 1980;303:897-902
- Phillips MD, Moake JL, Nolasco L, Turner N: Aurintricarboxylic acid: A novel inhibitor of the association of von Willebrand factor and platelets. *Blood* 1988;72:1898-1903
- Moake JL, Turner NA, Stathopoulos NA, Nolasco LH, Hellums JD: Involvement of large plasma von Willebrand factor (vWF) multimers and unusually large vWF forms derived from endothelial cells in shear stress-induced platelet aggregation. *J Clin Invest* 1986;78:1456-1461
- Moake JL, Turner NA, Stathopoulos NA, Nolasco L, Hellums JD: Shear-induced platelet aggregation can be mediated by vWF released from platelets, as well as by exogenous large or unusually large vWF multimers, requires adenosine diphosphate, and is resistant to aspirin. *Blood* 1988;71:1366-1374
- Weiss HJ, Hawiger J, Ruggeri ZM, Turitto VT, Thiagarajan P, Hoffmann T: Fibrinogen-independent platelet adhesion and thrombus formation on subendothelium mediated by glycoprotein IIb-IIIa complex at high shear rate. *J Clin Invest* 1989;83:288-297
- De Marco L, Girolami A, Russell S, Ruggeri ZM: Interaction of asialo von Willebrand factor with glycoprotein Ib induces fibrinogen binding to the glycoprotein IIb/IIIa complex and mediates platelet aggregation. *J Clin Invest* 1985;75:1198-1203
- Gralnick HR, Williams SB, Collier BS: Asialo von Willebrand factor interactions with platelets: Interdependence of glycoproteins Ib and IIb/IIIa for binding and aggregation. *J Clin Invest* 1985;75:19-25
- Folts JD, Gallagher K, Rowe GG: Hemodynamic effects of controlled degrees of coronary artery stenosis in short-term and long-term studies in dogs. *J Thorac Cardiovasc Surg* 1977;73:722-727
- Folts JD, Crowell EB Jr, Rowe GG: Platelet aggregation in partially obstructed vessels and its elimination with aspirin. *Circulation* 1976;54:365-370
- Mancini GBJ, Simon SB, McGillem MJ, LeFree MT, Friedman HZ, Vogel RA: Automated quantitative coronary arteriography: Morphologic and physiologic validation in vivo of a rapid digital angiographic method. *Circulation* 1987;75:452-460
- Back LD, Radbill JR, Crawford DW: Analysis of pulsatile, viscous blood flow through diseased coronary arteries of man. *Biomechanics* 1977;10:339-353
- Lipowsky HH, Usami S, Chien S: In vivo measurements of "apparent viscosity" and microvessel hematocrit in the mesentery of the cat. *Microvasc Res* 1980;19:297-319
- Clauss A: Rapid physiological coagulation method for the determination of fibrinogen. *Acta Haematol (Basel)* 1957;17:237-242
- Folts JD, Gallagher K, Rowe GG: Blood flow reductions in stenosed canine coronary arteries: Vasoconstriction or platelet aggregation. *Circulation* 1982;65:248-255
- Bertha BG, Folts JD: Inhibition of epinephrine-exacerbated coronary thrombus formation by prostacyclin in the dog. *J Lab Clin Med* 1984;103:204-214
- Eidt JF, Allison P, Noble S, Ashton J, Golino J, McNatt L, Buja LM: Thrombin is an important mediator of platelet aggregation in stenosed canine coronary arteries with endothelial injury. *J Clin Invest* 1989;84:18-27
- Collier BS, Folts JD, Scudder LE, Smith SR: Antithrombotic effect of a monoclonal antibody to the platelet glycoprotein IIb/IIIa receptor in an experimental animal model. *Blood* 1986;68:783-786
- Bellinger DA, Nichols TC, Read MS, Reddick RL, Lamb MA, Brinkhous KM, Evatt BL, Griggs TR: Prevention of occlusive coronary artery thrombosis by a murine monoclonal antibody to porcine von Willebrand factor. *Proc Natl Acad Sci USA* 1987;84:8100-8104
- Strony JT, Folts JD, Adelman B: In vivo antiplatelet effect of gly-arg-gly-aspartic-serine in the stenosed canine coronary artery occlusion model. *Clin Res* 1989;54:117a
- Shebuski RJ, Berry DE, Bennett DB, Romoff T, Storer BL, Ali F, Samamen J: Demonstration of ac-arg-gly-aspartic-NH₂

- as an antiaggregatory agent in the dog by intracoronary administration. *Thromb Haemost* 1989;61:183-189
21. Golino P, Buja LM, Ashton JH, Kulkarni P, Taylor A, Willerson JT: Effect of thromboxane and serotonin receptor antagonists on intracoronary platelet deposition in dogs with experimentally stenosed coronary arteries. *Circulation* 1988; 78:701-711
 22. Ashton JH, Schmitz JM, Campbell WB, Ogletree ML, Raheja S, Taylor AL, Fitzgerald C, Buja LM, Willerson JT: Inhibition of cyclic flow variations in stenosed canine coronary arteries by thromboxane A₂/prostaglandin H₂ receptor antagonists. *Circ Res* 1986;59:568-578
 23. Bush LR, Remy DC, Gaul SL: Effects of 3-methoxycycloheptadine in a canine model of coronary thrombosis. *J Pharmacol Exp Ther* 1988;247:796-802
 24. Bush LR, Campbell WB, Tilton GD, Buja LM, Willerson JT: Effects of the selective thromboxane synthetase inhibitor, dazoxiben, on cyclic flow variations in stenosed canine coronary arteries. *Trans Assoc Am Physicians* 1983;96:103-112

KEY WORDS • von Willebrand factor • aurointricarboxylic acid
• shear stress • platelets • platelet aggregation