Prevention of Occlusive Coronary Artery Thrombosis by Prostacyclin Infusion in the Dog

JOSEPH L. ROMSON, B.S., DAVID W. HAACK, PH.D., GERALD D. ABRAMS, M.D., AND BENEDICT R. LUCCHESI, PH.D., M.D.

SUMMARY The hemodynamic and antithrombotic properties of prostacyclin (PGI₂) were evaluated in an in vivo canine model in which left circumflex coronary artery (LCX) thrombus formation was initiated by electrical stimulation (150 \( \mu \text{A}, \text{DC for 6 hours} \)) of the artery's intimal surface via an implanted silver wire electrode. Eleven of the 12 control dogs (92\%) developed totally occlusive LCX thrombi after an average of 3.2 ± 0.4 hours of LCX stimulation; the remaining control dog underwent spontaneous ventricular fibrillation.

A PGI₂ infusion (150, 300 or 500 ng/kg/min) into the left atrial appendage was begun 10 minutes before the start of LCX stimulation and continued throughout the 6-hour stimulation period. LCX thrombus wet weight and the incidence of occlusive LCX thrombosis decreased in the PGI₂-treated dogs in a dose-dependent manner. Hemodynamically, after 6 hours of PGI₂ infusion at 500 ng/kg/min, mean arterial pressure decreased by 36 ± 4\%, cardiac output increased by 51 ± 14\%, and the effect on heart rate was inconsistent. Light and scanning electron microscopic examination of the LCX at the site of electrode insertion in PGI₂-treated dogs (500 ng/kg/min) revealed a damaged and denuded intimal surface but no thrombi, in contrast to the thrombus formation in similar specimens taken from control dogs. In this report, we describe the potent hemodynamic effects of prolonged PGI₂ infusion and demonstrate its ability to prevent coronary artery thrombosis in response to intimal injury.

NATURALLY OCCURRING prostaglandins have been intensively studied to characterize their complex effects on the cardiovascular system and to apply some of their unique pharmacologic properties to specific clinical cardiovascular problems. Prostacyclin (PGI₂) has been of particular interest since its discovery² because it can prevent³,⁴ or reverse⁵ in vivo platelet aggregation and has potent vasodilatory effects. In addition, investigations in the cat⁶,⁷ and the dog⁸ have demonstrated that PGI₂ has a protective effect on myocardium subjected to ischemia. The results of these and numerous other studies have prompted suggestions that PGI₂ may have clinical value in the management of specific cardiovascular diseases.

Recognition of the importance of circulating platelets in the pathogenesis of coronary artery thrombosis has stimulated the search for pharmacologic agents that can prevent extensive platelet aggregation in response to thrombogenic stimuli. The advantages of using a naturally occurring platelet-aggregation inhibitor led us to evaluate the in vivo antithrombotic properties of PGI₂. By modifying a recently published technique for inducing coronary artery thrombosis in the conscious dog,⁹ we could reliably induce totally occlusive left circumflex coronary artery (LCX) thrombosis within 3–4 hours in anesthetized, open-chest dogs. This experimental approach allowed us to evaluate the antithrombotic and hemodynamic impact of prolonged PGI₂ infusion. The results presented in this report demonstrate the marked antithrombotic and hemodynamic effects of PGI₂ in an animal model of coronary artery thrombosis.

Methods

Surgical Preparation

Thirty-seven male mongrel dogs that weighed 18–20 kg were used. Each dog was anesthetized with sodium pentobarbital (30 mg/kg, i.v.), intubated and placed on positive-pressure respiration. A left thoracotomy was performed at the fifth intercostal space and the pericardium was incised. An apical stab wound in the left atrial appendage permitted the insertion of a cannula (PE 260) used to infuse PGI₂. Distal to the atrial branch and proximal to its first descending branch, 1–2 cm of the LCX were isolated from surrounding tissue by careful blunt dissection. Within this isolated region, an electromagnetic flow probe was affixed to the artery for continuous measurement of LCX blood flow. To aid in the insertion of the LCX stimulating electrode, the tip of a 25-gauge hypodermic needle was secured to the leading end of the 28-gauge Teflon-coated silver wire. This electrode was inserted through the wall of the LCX so that the tip of the electrode (2–3 mm) was in contact with the intimal lining of the vessel. The electrode was connected in series to a 250,000-Ω potentiometer, a 9-V nickel-cadmium battery and a digital ammeter. The circuit was completed by suturing a disc electrode to a subcutaneous region on the chest wall. The stimulating circuit was designed to permit constant monitoring and easy adjustment of the direct anodal current delivered to the intimal surface of the LCX.

Hemodynamic data were recorded continuously during the course of the experiment on a six-channel
Grass polygraph. Arterial blood pressure was measured with a Statham pressure transducer attached to a polyethylene catheter advanced to the abdominal aorta by way of the right femoral artery. Epicardial electrograms were obtained by suturing a monopolar electrode in the region of the left ventricular myocardium perfused by the LCX. Segmental left ventricular isometric contractile force in the region of the myocardium dependent on LCX flow was measured with a Brodie-Walton strain-gauge arch. Replicate thermodilution cardiac output determinations were made every 30 minutes throughout the experiment. After completion of the surgical preparation, each dog received a supplemental dose of sodium pentobarbital (5 mg/kg, i.m.) to maintain a stable level of anesthesia. Thirty minutes were allowed at the conclusion of the surgical preparation for each dog to stabilize.

Initiation of PGI₂ Infusion and LCX Stimulation

After basal hemodynamic measurements were recorded, a continuous infusion of PGI₂ (150, 300 or 500 ng/kg/min) or drug diluent (50 mM isotonic Tris-HCl buffer, pH 9.4) at 4°C was started into the left atrial appendage. The constant infusion rate of 0.5 ml/min was maintained by a peristaltic pump, which was calibrated at the start of each experiment. PGI₂ solutions in 50 mM isotonic Tris-HCl (pH 9.4) were made daily from crystalline sodium PGI₂ and maintained on ice (0-4°C) during the 6-hour infusion. Ten minutes after the start of infusion, electrical stimulation of the LCX was initiated at 150 μA via the implanted silver wire electrode. Stimulation of the LCX and left atrial infusion of PGI₂ or Tris buffer were continued for 6 hours. Immediately before sacrifice by electrical fibrillation, a 20% patent blue violet solution (1 ml/5 kg body weight) was injected into the left atrial appendage. Introduction of this flow-dependent dye into the myocardial circulation permitted post-mortem quantification of the extent of left ventricular myocardium that was not receiving coronary blood flow as a result of LCX coronary artery thrombosis.

After removal of the heart, the LCX was dissected free of surrounding tissue and opened lengthwise. Adhering thrombotic material was removed from the intimal surface of the LCX to determine thrombus wet weight. The heart was sectioned transversely from apex to base in 1.0-cm-thick rings. The nonperfused regions of the left ventricular myocardium, identified by the absence of patent blue violet, were dissected free of surrounding myocardium and weighed. The amount of the nonperfused tissue is expressed as a percent of the total left ventricular mass.

LCX Microscopy

LCX samples for light microscopy were prepared by fixation for 2 hours at room temperature in a solution of 2.5% glutaraldehyde (V/V) and 2% paraformaldehyde (W/V) in 0.1 M cacodylate buffer (pH 7.4). The samples were then transferred to 10% sucrose in 0.1 M cacodylate buffer (pH 7.4) and stored at 4°C. Samples to be examined by scanning electron microscopy were postfixed for 1 hour in 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.4), dehydrated in a graded series of ethanol solutions and desiccated by critical-point drying. After the samples had been coated with gold, they were examined with an AMR 1200 scanning electron microscope.

Statistics

Except where otherwise noted, all data were analyzed using an analysis of variance software package available through the Michigan Terminal System. A p value less than 0.05 was considered significant. Data are expressed as the mean ± SEM unless noted otherwise.

Results

Low-amperage electrical stimulation of canine LCX consistently produces occlusive coronary artery thrombosis in dogs receiving a drug-free infusion of Tris buffer (controls). In this report, occlusive LCX thrombosis is determined primarily on the basis of total cessation of LCX blood flow. The time when thrombotic occlusion of the vessel occurred is readily determined by continuously monitoring LCX blood flow. A typical sequence of the hemodynamic changes associated with thrombotic occlusion of the LCX in control dogs is shown in figure 1. Complete cessation of LCX blood flow occurred after 1 hour and 15 minutes of stimulation. However, signs of developing ischemia are evident as early as 45 minutes into the stimulation period. At this time, a reduction in left ventricular isometric force in the LCX perfused region of myocardium, an alteration in the phasic coronary blood flow wave form and a reduction in mean coronary blood flow are evidence that LCX thrombosis is occurring. After 47 minutes of stimulation, ST-segment elevation can be seen in the epicardial electrode situated in the LCX dependent region of the myocardium. In addition, left ventricular force in this region is further diminished. Complete LCX occlusion by intravascular thrombosis occurs at 1 hour and 15 minutes of LCX coronary artery stimulation. Further elevation of the epicardial electrogram ST segments appear as LCX flow is reduced to zero. Isometric left ventricular contractile force, as measured by the Brodie-Walton strain gauge, is markedly altered. Instead of active development of force, the ischemic myocardium is undergoing passive bulging in response to intraventricular pressure increases occurring during systole (at 1 hour, 15 minutes). The average time to complete LCX occlusion in the control dogs was 3.2 ± 0.4 hours of LCX stimulation. Although the complete cessation of LCX blood flow in figure 1 occurred after 1 hour and 15 minutes of LCX stimulation, the changes shown in this figure are representative of the control dogs.

Sequential sampling of a continuous record from a dog receiving PGI₂ infusion (500 ng/kg/min) during
the 6-hour stimulation period is presented in figure 2. The most notable effect of PG12 at this dose is the marked reduction in arterial blood pressure. The epicardial electrogram remained normal over the entire stimulation period and mean LCX blood flow was increased. Left ventricular isometric force declined slightly over the course of the experiment. In short, infusion of PG12 prevented occlusive LCX thrombosis, avoiding the ischemia-induced deterioration of regional myocardial performance seen in figure 1.

Figure 3 indicates the changes in LCX blood flow in each dog of the control and PG12 (500 ng/kg/min) treatment groups. In the control dogs, LCX blood flow steadily declined until there was complete cessation of blood flow through the coronary artery. Eleven of the 12 control animals (92%) developed occlusive thrombi; the other dog in the group developed spontaneous ventricular fibrillation 4 hours into the experiment. The average time to complete thrombotic occlusion of the LCX, based on these coronary blood flow data, was 3.2 ± 0.4 hours (range 1.25–5 hours). Infusion of PG12 at a dose of 500 ng/kg/min prevented the decline and eventual cessation of LCX blood flow characteristic of the control dogs.

The hemodynamic effects of a 6-hour infusion of PG12 at doses of 150, 300 or 500 ng/kg/min are summarized in figure 4. The data in the lower left corner of figure 4 show the mean changes in LCX blood flow for each of the four treatment groups. In the control dogs, coronary blood flow progressively declined. Infusion of PG12 (150, 300 or 500 ng/kg/min) prevented the reduction in LCX blood flow seen in the control dogs.

The dose-dependent reduction in arterial blood pressure was rapid, with the maximal effect occurring within 10 minutes after the initiation of infusion (fig. 4). Arterial blood pressure remained depressed for the entire period of PG12 infusion. After 6 hours of PG12 infusion at a dose of 500 ng/kg/min, mean arterial pressure was reduced by 36 ± 4% relative to the preinfusion basal readings. Upon termination of the infusion, mean arterial pressure returned toward basal levels.

Cardiac output was increased by 51 ± 14% over basal readings after 6 hours of infusion of PG12 at a dose of 500 ng/kg/min (fig. 4). PG12 infusion at 300 ng/kg/min significantly increased cardiac output. No dose-dependent relationship was found between PG12 and heart rate. At a dose of 300 ng/kg/min of PG12, heart rate increased, but at doses of 150 and 500 ng/kg/min, the heart rates were not significantly different from those of control dogs. The increase in heart rate seen in control dogs between 4 and 6 hours is probably the result of enhanced sympathetic activity in response to regional myocardial ischemia produced by coronary artery thrombosis. Whereas the PG12-induced alterations in heart rate are relatively small and inconsistent and no increase in left ventricular isometric force was seen, the marked increase in cardiac output is attributed mainly to the reduction in outflow impedance resulting from the hypotensive properties of PG12.

**Antithrombotic Effects of PG12**

Postmortem light and scanning electron microscopic examination of the LCX from control and PG12-treated dogs show the in vivo antithrombotic
properties of PGI₂. Figure 5 is a light micrograph of two LCX arteries, one from a dog that received drug-free Tris buffer infusion and the other from a PGI₂-treated dog (500 mg/kg/min). The arteries were sectioned transversely, immediately distal to the site at which the electrode was introduced into the lumen of the artery. The lumen of the vessel from the control dog is distended and completely filled with thrombotic material deposited in response to intimal injury caused by 6 hours of electrical stimulation of the LCX. The LCX blood flow in this dog showed changes that were characteristic of the control dogs, with thrombotic occlusion of the LCX occurring after 3.5 hours of electrical stimulation. A transverse section of an LCX from the PGI₂-treated dog is shown in the lower portion of figure 5. Directly above the electrode site is a dark thrombus mass that occupies less than 50% of the arterial lumen. During fixation and sectioning, the

Figure 2. A sequence of recordings from a representative experiment in which PGI₂ (500 ng/kg/min) was infused into the left atrial appendage for 6 hours. Definitions of the tracings are as in figure 1.

Figure 3. Changes in left circumflex coronary artery (LCX) blood flow for each dog in the control and prostacyclin (500 ng/kg/min) treatment groups. Complete cessation of LCX blood flow as a result of thrombotic occlusion of the vessel is indicated by lines that intercept the abcissa. Lines that terminate before the 6-hour time point indicate spontaneous ventricular fibrillation in that dog.
vessel partially collapsed. This dog showed no significant change in coronary blood flow.

Light and scanning electron micrographs of the luminal surface of the LCX coronary artery from control and PGI₂-treated dogs (500 ng/kg/min) are shown in figures 6 and 7. The coronary arteries were prepared by opening the vessel longitudinally, revealing the lumen of the LCX at the site of electrode insertion. Figure 6A shows an occlusive thrombus mass within the lumen of an LCX removed from a control dog. Distal to the site of electrode insertion (asterisk) the thrombus is lightly colored and marked with interspersed, dark transverse bands. A scanning electron micrograph of the region of the thrombus indicated by the asterisk is shown in figure 6B. Large platelet aggregates, fibrous material and trapped erythrocytes can be seen in this high-power field. The proximal portion of the thrombus (fig. 6A) is much darker. This region is composed almost entirely of erythrocytes (red thrombus), resulting from stasis of blood flow. Platelet aggregates adhering to the damaged endothelium of the vessel and thrombus formation caused enough disruption of coronary blood flow to permit stasis coagulation.

Figure 7A, a low-power scanning electron micrograph of the luminal surface of the LCX from a dog receiving PGI₂ (500 ng/kg/min), reveals the potent antithrombotic properties of PGI₂. After 6 hours of electrical stimulation of the LCX, there is a lesion on the intimal surface, but little thrombotic material has been deposited. Injury of this magnitude would be expected to induce platelet aggregation and thrombus formation. Figure 7B reveals many erythrocytes trapped in a meshwork of fibrous material. The platelet aggregates seen in figure 6B are missing in this field. The intimal surface distal to the site of electrode

**FIGURE 4.** The hemodynamic effects of a 6-hour PGI₂ infusion. Data are expressed as an average percent change relative to basal recordings obtained before the drug infusion. The average time to occlusion (lower left) indicates the mean time at which the dogs in the control group developed occlusive left circumflex coronary artery (LCX) thrombi (see top of figure 3). Significant differences (p < 0.05) relative to the data from animals receiving Tris buffer (vehicle) are indicated by an asterisk. The infusion dosage and number of dogs in each group are indicated to the right of the graph.
Figure 6. Micrographs of a left circumflex coronary artery (LCX) removed from a control dog. (A) Light micrograph of the occlusive thrombus mass contained within the lumen of the LCX, which developed in response to intimal injury produced by 6 hours of LCX stimulation at 150 μA. The asterisk indicates the site at which the stimulating electrode penetrates the arterial wall. (B) A high-magnification scanning electron micrograph from the region indicated by the asterisk in panel A displays large platelet aggregates, fibrous material and trapped erythrocytes.

The antithrombotic properties of PGI₂ in this in vivo model of coronary artery thrombosis are summarized in figure 8. As the dose of PGI₂ is increased from 150 to 500 ng/kg/min, the number of dogs in each treatment group that develop occlusive thrombi decreases. At 150 ng/kg/min, 50% occlude; at 300

Figure 7. Scanning electron micrographs of a left circumflex coronary artery (LCX) removed from a dog that received a 6-hour infusion of PGI₂ (500 ng/kg/min). (A) Low-power micrograph of the luminal surface of the LCX at the site of electrode insertion. Six hours of electrical stimulation of the intimal surface at 150 μA produced the ulcerous lesion. The lumen is essentially free of thrombotic material. (B) A higher-magnification view of the region indicated by the arrow in panel A. While many erythrocytes are trapped in a meshwork of fibrous material, there are no large platelet aggregates as in figure 6B. (C) View of the intimal surface of the LCX distal to the site of the electrode indicated by the asterisk in panel A. The normally smooth endothelial surface has been disrupted, revealing subintimal structures. However, little platelet aggregation is seen.
infusion in an in vivo model of coronary artery thrombosis. Folts et al.\textsuperscript{11} developed a technique for producing cyclic alterations in canine LCX blood flow due to platelet aggregates by narrowing the lumen of the vessel with a plastic occluder. Using this technique, Aiken et al.\textsuperscript{13} demonstrated a reduction in the frequency of occlusive episodes with PGI\textsubscript{2} infusion, indicating that in vivo platelet aggregation is inhibited by PGI\textsubscript{2}.

Our model may more realistically simulate human coronary artery thrombus formation, as intimal injury produced by low-amperage electrical stimulation of the LCX induces platelet adhesion and secondary recruitment of platelets to form aggregates. The initial platelet thrombus is stabilized by incorporating fibrin into the growing thrombus mass. The thrombus has an intravascular mass similar in morphology and composition to human coronary artery thrombi (fig. 6).\textsuperscript{13} Thus, the reported method of inducing coronary artery thrombosis provides a more appropriate model to study the antithrombotic properties of compounds like PGI\textsubscript{2}.

In this animal model, constant infusion of PGI\textsubscript{2} at a dose of 500 ng/kg/min inhibited in vivo platelet aggregation and consistently prevented thrombotic occlusion of the LCX at the site of electrically induced intimal injury. Eleven of 12 control dogs (92%) and one of 11 PGI\textsubscript{2}-treated dogs (500 ng/kg/min) (9%) developed occlusive LCX thrombi. Cessation of LCX blood flow during the course of the experiment was used as an indication of thrombotic occlusion. Postmortem examination of the LCX revealed that in dogs in which cessation of LCX coronary blood flow occurred, intravascular thrombi completely filled the arterial lumen. However, in those animals in which LCX coronary blood flow was unaltered over the course of the experiment, nonocclusive mural thrombi were found (fig. 5). This progressive decline and ultimate cessation of LCX blood flow correlates with complete thrombotic filling of the vascular lumen.

The vasodilating properties of PGI\textsubscript{2} may have played a role in maintaining LCX blood flow by relaxing vascular tone, permitting blood to flow between the arterial wall and thrombus mass. However, in a series of preliminary experiments, a brief infusion of PGI\textsubscript{2} (500 ng/kg/min) after thrombotic occlusion of the LCX was never observed to increase blood flow through the vessel (unpublished observations). In addition, the marked differences noted between PGI\textsubscript{2}-treated and control animals with respect to intravascular thrombus mass (fig. 8) and morphology (figs. 5–7) suggest that PGI\textsubscript{2} prevents occlusion of the LCX primarily by inhibiting thrombus formation at the site of intimal injury.

The hemodynamic effects of PGI\textsubscript{2} when administered as a 6-hour infusion are, for the most part, similar to results after a short exposure to the drug.\textsuperscript{15, 16} PGI\textsubscript{2} is a potent vasodilator and produces a dose-dependent reduction in arterial blood pressure. Other investigators\textsuperscript{15, 16} have reported inconsistent effects of PGI\textsubscript{2} on heart rate and cardiac output. In the present report, a paradoxical bradycardia induced by a high dose of PGI\textsubscript{2} (500 ng/kg/min) was seen over...
the initial 10 minutes of infusion. However, the trend over the 6-hour infusion period was toward elevated heart rates. The pronounced increases in cardiac output with PG1 infusion in this study differ somewhat from the inconsistent effects reported elsewhere. The primary difference between these reports is the method of administration of PG1. Before the present report, most investigators have relied on bolus injections or short-term infusions of PG1. The continuous infusion of PG1 over 6 hours permitted the experimental preparation to stabilize from the drastic initial hemodynamic effects of PG1 administration. In summary, the prolonged, constant infusion of PG1 at various dosage levels provided considerable information on the effects of PG1 on various hemodynamic measurements.

Our primary concern in this study was to evaluate the antithrombotic properties of PG1. However, we felt it was more appropriate to study the hemodynamic impact of prolonged PG1 infusion during active coronary artery thrombosis than to study the agent in an experimental model in which the coronary circulation remains unperturbed. Despite the complications introduced into the interpretation of hemodynamic data obtained during coronary artery thrombosis and subsequent regional myocardial ischemia, this experimental approach more closely parallels the clinical setting in which PG1 may be used in the management of human coronary artery thrombosis. Our data indicate that PG1 is not the ideal therapy for treatment of arterial thromboembolic conditions. Chemical instability and short biologic half-life demand careful attention to formulation conditions (pH 9.4 at 4°C) and constant infusion to achieve a desired antithrombotic effect. In addition, the marked vasodilatory actions of PG1 have a significant impact on cardiac hemodynamics. The potent vasodepressor effects of PG1 may be limiting, especially in patients with coronary artery disease in whom a reduction in coronary perfusion pressure may precipitate myocardial ischemia. In this study, a dose of 500 ng/kg/min was necessary to reliably prevent coronary artery thrombosis. This dose resulted in a dramatic reduction in arterial blood pressure. A dose of 50 ng/kg/min has produced signs of cardiovascular collapse in healthy volunteers. However, to facilitate the use of this experimental model, we had to induce occlusive thrombus development over a reasonably short period. Thus, the stimulus necessary to reproducibly induce this occlusive event would a priori be more drastic than naturally occurring arterial thrombogenic stimuli, and logically, would require more aggressive pharmacologic intervention.

Nevertheless, PG1 may have considerable therapeutic value. In addition to its antithrombotic actions, recent data indicate a protective effect on ischemic myocardium. Platelets have been postulated to play a role in the formation of arteriosclerotic lesions, and there have been reports of a reduction in vascular PG1 synthesis in arteriosclerotic vessels. This may suggest that PG1 can be used as a prophylactic treatment against progressive arteriosclerosis. One of the earliest reports of successful clinical application of PG1 in treating peripheral vascular disease came from Szczeklik et al. Five patients with advanced arteriosclerosis obliterans experienced a significantly improved clinical status with prolonged PG1 infusion.

Two general schemes may be anticipated for improving the clinical acceptability of PG1. First, the concomitant use of a phosphodiesterase inhibitor, such as theophylline or dipryramide, may be expected to potentiate the effect of PG1 in inhibiting platelet aggregation by maintaining elevated intracellular platelet cAMP levels. Evidence obtained in vitro and in the rabbit indicate that phosphodiesterase inhibitors prolong the platelet inhibitory and antithrombotic properties of PG1, respectively. Preliminary, unpublished data in the model detailed in this report indicate that the concomitant use of aminophylline as a phosphodiesterase inhibitor permits a 10-fold reduction in the amount of PG1 necessary to prevent coronary artery thrombosis. Thus, one may significantly reduce the dose of PG1 and, in turn, reduce its adverse hemodynamic impact while still maintaining its antithrombotic actions.

The second approach toward improving the clinical value of PG1 would be the development of stable PG1 analogs. Many investigators are attempting to manipulate the structure of PG1 to enhance its biologic stability. However, for the most part, PG1 analogs that possess prolonged in vitro antiplatelet aggregating activity generally are as short-lived as natural PG1 in vivo. But it appears to be only a matter of time before a useful PG1 analog with in vivo platelet inhibitory properties and minimal vasodepressor effects is developed and used to treat human thrombotic disorders.

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