Roles of Thrombin and Platelet Membrane Glycoprotein IIb/IIIa in Platelet-Subendothelial Deposition After Angioplasty in an Ex Vivo Whole Artery Model

Aaron V. Kaplan, MD; Lawrence L.-K. Leung, MD; Wing-Hung Leung, MB, MRCP; Gordon W. Grant, BS; I. Ross McDougall, MB, ChB, PhD; and Tim A. Fischell, MD

Background. Platelet deposition at the site of injury caused by balloon angioplasty is associated with acute closure and restenosis.

Methods and Results. In a new ex vivo whole artery angioplasty model, we examined the roles of thrombin inhibition with $\text{d-Phe-Pro-Arg} CH_2 Cl$ (PPACK) and inhibition of the platelet membrane fibrinogen receptor glycoprotein IIb/IIIa (GPIIb/IIIa) with monoclonal antibody 7E3 on platelet deposition at the site of balloon injury. Fresh rabbit aortas were mounted in a perfusion chamber. One half of the mounted arterial segment was dilated with a standard angioplasty balloon catheter and the uninjured half served as the control segment. The vessels were perfused with human blood at physiological pressure and shear rates of 180–250 sec$^{-1}$ for 30 minutes. Platelet deposition was measured using $^{111}$In-labeled platelets and scanning electron microscopy. With heparin (2 units/ml) anticoagulation, $8.2 \pm 2.2 \times 10^4$ platelets/cm$^2$ were deposited at the site of balloon injury compared with $0.7 \pm 0.2 \times 10^4$ platelets/cm$^2$ on uninjured segments ($p<0.02, n=7$). PPACK was tested at a concentration (10 $\mu$M) that totally inhibited platelet aggregation in response to thrombin. 7E3 was tested at a concentration (10 $\mu$g/ml) that totally inhibited platelet aggregation. Platelet deposition at the site of balloon injury was reduced 47% by PPACK and 70% by 7E3 compared with heparin.

Conclusions. At shear rates seen in nonstenotic coronary arteries, PPACK and 7E3 are more effective than heparin in reducing platelet deposition at the site of balloon injury. The significant inhibition of platelet deposition by PPACK demonstrates the importance of heparin-resistant thrombin in platelet thrombus formation. The 7E3 results suggest that approximately 70% of platelet deposition at the site of balloon injury is GPIIb/IIIa dependent and that the remaining 30% results from non–GPIIb/IIIa-mediated platelet-subendothelial adhesion. Finally, the ex vivo whole artery system is a useful model for studying platelet–vessel wall interactions under physiologically defined parameters. (Circulation 1991;84:1279–1288)

Platelet deposition at the site of arterial injury may play an important role in acute occlusion and restenosis after percutaneous transluminal coronary angioplasty (PTCA). Platelet thrombus formation is observed soon after angioplasty.\textsuperscript{1} PTCA disrupts the antithrombotic endothelial cell barrier and causes deep arterial wall injury, exposing subendothelium, media, and atheromatous to blood elements. This injury provides a strong stimulus for platelet activation and thrombus formation. It has

A.V.K. is a recipient of National Research Service Award HL-08271 from the National Institutes of Health. L.L.-K.L. is an Established Investigator of the American Heart Association. T.A.F. is a Clinical Investigator of the National Heart, Lung, and Blood Institute (grant HL-02001).

Address for correspondence: Aaron V. Kaplan, MD, Falk Cardiovascular Research Center, Stanford University School of Medicine, Stanford, CA 94305.

Received September 17, 1990; revision accepted April 30, 1991.
been postulated that platelet deposition at the site of balloon-induced arterial injury is one of the initiating events leading to restenosis.\textsuperscript{2,3} Platelets are known to release potent growth factors (e.g., platelet-derived growth factor) that are capable of stimulating myointimal proliferation.\textsuperscript{2,3} The atherosclerotic lesions induced by balloon injury can be prevented in rabbit and rat models by thrombocytopenia produced by a polyclonal antiplatelet serum.\textsuperscript{4,5} These data suggest a potential link between platelet deposition at the site of PTCA injury and subsequent restenosis. Although several models have been described to study platelet-vessel wall interactions, none is well suited to examine interactions between balloon angioplasty-induced injury and human platelets.\textsuperscript{6,7} To better define the factors leading to platelet thrombus formation after angioplasty, we developed an ex vivo whole artery model to study platelet-vessel wall interactions under physiological conditions at specific shear rates.

Thrombin may be the most potent in vivo platelet activator. Thrombin is generated at the site of balloon injury as a result of disruption of the endothelial cell surface with release of tissue factor and exposure of the subendothelial matrix to circulating blood. D-Phe-Pro-ArgCH\textsubscript{2}Cl (PPACK) is a synthetic oligopeptide that inhibits thrombin by irreversibly binding to the serine protease active site.\textsuperscript{8} PPACK inhibits platelet thrombus formation on Dacron arterial grafts and on carotid arteries after endarterectomy in baboons.\textsuperscript{6,9} Heparin, an acidic sulfated mucopolysaccharide, inhibits thrombin primarily by potentiating the effects of antithrombin III (ATIII) and secondarily by the inactivation of coagulation factors IX, X, XI, and XII.\textsuperscript{10} Heparin has been shown to be relatively ineffective compared with other thrombin inhibitors in decreasing platelet thrombus formation on Dacron graft material in the baboon and on superficially injured porcine carotid arteries after PTCA.\textsuperscript{6,11}

Platelet thrombus formation at the site of balloon injury is the result of platelet-vessel wall and platelet-platelet interactions. The platelet membrane fibrinogen receptor glycoprotein IIb/IIIa (GP\textsubscript{IIb/IIIa}) is the mediator of the initial stage (primary phase) of platelet aggregation. GP\textsubscript{IIb/IIIa} is the most abundant protein receptor found on platelet membranes (∼50,000 complexes per platelet). After activation, GP\textsubscript{IIb/IIIa} binds to fibrinogen, resulting in the cross linking of platelets.\textsuperscript{12-14} The murine monoclonal anti-GP\textsubscript{IIb/IIIa} antibody 7E3 is one of the most potent antiplatelet agents available. 7E3 completely inhibits platelet aggregation in vitro and has been demonstrated to prevent coronary reocclusion after reperfusion with thrombolytic agents in an animal model.\textsuperscript{15,16}

In the present study, we compared the effects of heparin, PPACK, and 7E3 on platelet deposition in an ex vivo model. The purpose was to determine the relative role of thrombin and GP\textsubscript{IIb/IIIa} in mediating platelet thrombus formation at the site of balloon injury.

### Methods

Freshly harvested rabbit aortas were mounted in a perfusion chamber. One half of the intact vessel was subjected to balloon angioplasty, and then the entire segment was perfused with human blood at physiological pressure and flow. Quantitative measurements of platelet deposition in the control and injured arterial segments were performed using \textsuperscript{11}In-labeled platelets. Qualitative assessment of platelet deposition was examined using scanning electron microscopy.

#### Flow Chamber

The flow chamber used is a modification of the chamber described by Fischell et al\textsuperscript{17} (Figure 1). The flow chamber comprises an outer warming bath and an inner vessel chamber. Vessels were mounted around a connector piece that allowed access to the vessel lumen for introduction of angioplasty catheters as well as for perfusion. The perfusate, physiological saline (see below), or whole blood was delivered at specific flow rates by a peristaltic pump (Masterflex Digistaltic Pump, Cole-Palmer, Barrington, Ill). Perfusion pressure was monitored by a fluid-filled transducer placed proximal to the mounted artery. At a chosen flow rate, the infusion pressure was regulated by minor adjustments in the outflow resistance. Nonthrombogenic materials were used for tubing and connectors (i.e., Lucite, silicon, and polyethylene).

#### Vessel Preparation

All animal studies were performed with the approval of the Stanford University Administrative Panel on Laboratory Animal Care in accordance with federal guidelines. New Zealand White rabbits were killed by cerebral concussion. The thoracic aortas were excised. The segments were then gently rinsed in physiological saline and mounted in the bath as shown (Figure 1). A careful dissection of adherent connective tissue and fat was performed. Intercostal arteries arising from the aorta were identified and ligated by silk suture or electrocautery. During the dissection, the vessel was gently perfused (less than 15 mm Hg and less than 15 ml/min) with physiological saline solution composed of (mM) NaCl 118, KCl 4, MgSO\textsubscript{4} 1.2, CaCl\textsubscript{2} 2, dextrose 5, NaHCO\textsubscript{3} 24, and NaH\textsubscript{2}PO\textsubscript{4} 1.2 (pH 7.40–7.45). The perfusion chamber was aerated with 95% O\textsubscript{2}-5% CO\textsubscript{2} and warmed to 37°C via the outer heating bath.

A conventional balloon angioplasty catheter (5.0-mm diameter and 2-cm length for thoracic aortas) was passed into the proximal portion of the vessel. Three 30-second balloon inflations at 6 atm were performed. The location of the catheter tip was marked, and the arterial segment distal to the tip was designated as the uninjured “negative” control segment.
Ultrasonic Measurements

During perfusion of blood, vessel diameter was measured by two-dimensional ultrasonic imaging as previously described. A 10-mHz transducer (Diasonics 200 RF, Milpitas, Calif.) was positioned approximately 1 cm above the artery in contact with the bath solution. The transducer position was adjusted manually to optimize the long-axis image. The vessel diameter was measured using a manually adjusted caliper system contained in the software package within the ultrasound machine. Both injured and control segments were measured twice during perfusion at three points approximately 2 mm apart. The mean of these six measurements was used for calculations of density of platelet deposition and shear rate.

Preparation of Blood

After obtaining informed consent and with the approval of the Stanford University School of Medicine Administrative Panel on Human Subjects in Medical Research, blood was obtained from healthy adult volunteers who did not smoke cigarettes and had not taken aspirin for at least 10 days or any other medication for at least 3 days. Venous blood was drawn via a 19-gauge butterfly needle using a blood pressure cuff at 60 mm Hg. A double-syringe technique was used in which 3 ml of blood was withdrawn into the first syringe, which was discarded, and 27 ml of blood to be used experimentally was withdrawn into the second syringe, which contained 3 ml of physiological saline solution with test reagent.

\[ ^{111}\text{In Labeling} \]

Platelets were labeled with \(^{111}\text{In-oxine} \) following the technique of Thakur et al. Platelets were harvested from 21 ml of human whole blood drawn into 3 ml of 0.9\% sodium citrate. Platelet-rich plasma was obtained by spinning the blood at 120g for 10 minutes. The platelet-rich plasma was then spun at 1,100g for 10 minutes. The platelet-poor plasma was decanted. The platelet pellet was gently washed and resuspended in 4 ml of calcium-free Tyrode’s solution. The calcium-free Tyrode’s solution used was the solution described by Thakur et al without heparin and with the addition of apyrase 100 mg/l (Sigma Chemical Co., St. Louis, Mo.). Then, 100 \( ^{111}\text{In-oxine} \) (Amersham Corp., North Chicago, Ill.) was added to the platelet suspension and incubated for 10 minutes at 37°C. Five milliliters of platelet-poor plasma was then added to the platelet suspension. This suspension was centrifuged at 1,100g for 10 minutes, and the platelet pellet was resuspended in 1 ml of platelet-poor plasma. Labeling efficiency was determined by dividing the activity in the resuspended pellet by the activity of the \(^{111}\text{In-oxine} \) initially added. The preparation was used only if the efficiency was more than 20\%.

Arterial Perfusion

Whole blood with \(^{111}\text{In-labeled} \) platelets admixed was perfused through the vessel at physiological pressure (mean pressure, 40–60 mm Hg) for 30 minutes (unless otherwise stated). The flow rate was adjusted to achieve a shear rate of 180–250 second\(^{-1} \). This shear rate was chosen because it is within the range seen in nonstenotic epicardial coronary arteries. The artery was then gently washed by perfusion with physiological saline (less than 15 mm Hg and less than 15 ml/min) until the perfusate was grossly clear (approximately 5
minutes). The vessel was then fixed by perfusion with isotonic glutaraldehyde solution at the same low pressure and flow rates for 10 minutes. The arterial segment was removed from the chamber, and sections were cut using a pair of single-edged razor blades mounted in parallel and set 3 mm apart. The segments were then placed into 1 ml of isotonic glutaraldehyde solution and counted in a gamma counter.

Scanning Electron Microscopy

Scanning electron microscopy was performed by standard techniques. Arterial segments were fixed in 3% glutaraldehyde in 0.1 M cacodylate buffer. Specimens then underwent acetone dehydration and critical point drying with CO\textsubscript{2}. After coating with gold-palladium, the segments underwent scanning. Representative areas were then photographed. Sections were also submitted for routine hematoxylin and eosin staining.

Platelet Aggregometry

Platelet aggregation was performed with platelet-rich plasma in the standard manner using a Chronolog dual-channel aggregometer (Chrono-Log Corp., Havertown, Pa.). Human thrombin (gift of Marc Shuman, MD, University of California at San Francisco) and ADP and collagen (Chrono-Log) were used as platelet agonists.

Test Agents

PPACK was obtained from Calbiochem (San Diego, Calif.). Beef lung heparin was obtained from Upjohn Co. (Kalamazoo, Mich.). Monoclonal antibody 7E3 was generously provided by Barry S. Coller, MD, State University of New York at Stony Brook.

PPACK was studied at a concentration of 10\textsuperscript{−3} M, which is 100-fold greater than the IC\textsubscript{50}. This concentration was chosen to ensure total thrombin inhibition. The effect of PPACK (10\textsuperscript{−4} M) on platelet aggregation was tested by in vitro aggregometry. Similarly, 7E3 was studied at a concentration of 10 \mu g/ml to ensure maximal inhibition of GP\textsubscript{IIb/IIIa}. The inhibitory effect of 7E3 was also tested in vitro by platelet aggregation studies performed on platelet-rich plasma from blood after perfusion through the ex vivo model. The heparin dose of 2 units/ml whole blood was chosen to achieve maximal activation of plasma ATIII as demonstrated by marked prolongation of the activated partial thromboplastin time of more than 120 seconds.

Computation

All data are given as platelets/per centimeter squared of arterial luminal surface as determined by the following formula:

\[
\frac{\{\text{Segment counts per minute} - \text{background}\}}{\text{(platelets/count)}} \div \text{Vessel surface area (cm}^2)\]

where platelets/per count was determined by dividing the platelet count by counts per minute from 1 ml of blood, and vessel surface area was calculated from measured vessel length and diameter.

Shear rates were calculated for each arterial segment using the following formula:

\[
\text{Shear rate} = \frac{4(\text{flow})}{\pi(\text{radius})^3}
\]

The results of experiments performed on different days with different donors are pooled. In each experiment for each test group, an average of three vessel segments underwent gamma counting. Results are normalized using platelet deposition with heparinized blood as 100% deposition.

The two-sided, paired Student's t test was used to compare the effect of different test agents on platelet deposition. A probability value of less than 0.05 was considered statistically significant. Values are reported as mean±SEM unless otherwise stated.

Results

Microscopic Assessment of Platelet Thrombus Formation and Vascular Injury

Scanning electron microscopy of the luminal surface of rabbit aortas after perfusion with human blood was carried out to document the extent of vascular injury and platelet thrombus formation (Figure 2). The uninjured (control) arterial segments showed intact endothelial cells without platelet deposition (Figure 2A). In contrast, the balloon-injured segments showed complete endothelial denudation with platelet thrombi deposition (Figure 2B). Light microscopy was performed to evaluate the extent of the injury induced by balloon inflations. Transverse sections of balloon-injured arterial segments revealed endothelial denudation without tears into the subintima or media (Figure 3B); uninjured segments showed an intact endothelial layer (Figure 3A).

Time Course of Platelet Deposition

To determine the time course of platelet deposition after balloon injury, segments were perfused with citrated human blood for varying periods of time. Platelet deposition on the injured arterial segment was time dependent and reached a plateau at 30–45 minutes with 4.18±0.28×10\textsuperscript{6} platelets/cm\textsuperscript{2} (Figure 4). This magnitude of platelet deposition is in agreement with other reported studies. Based on these data, a perfusion time of 30 minutes was chosen for the PPACK and 7E3 studies to ensure that the maximal effect was observed.

Platelet aggregation studies were performed to examine the effect of perfusion through the ex vivo system on platelet function. Platelet aggregation in response to varying concentrations of ADP (0.25–10 \mu M) was examined with platelet-rich plasma from blood perfused through injured and uninjured arterial segments and compared with control (nonper-
fused) blood. No significant differences in platelet aggregation were observed in terms of sensitivity of platelets to threshold doses of agonist and maximal aggregatory responses (data not shown). These results indicate that perfusion through the system did not cause significant platelet activation.

**Effect of PPACK, 7E3, and Heparin on Platelet Aggregation**

When added to citrated platelet-rich plasma (10^{-3} M), PPACK completely inhibited thrombin-induced platelet aggregation (Figure 5). Similarly, the inhibitory effect of 7E3 was confirmed in vitro.

**Figure 2.** Scanning electron photomicrographs of injured and uninjured segments showing luminal surface of a rabbit aorta after perfusion with human blood (original magnification, ×1,300). Panel A: Representative area of uninjured (control) segment demonstrating intact endothelial cells without platelet deposition. Panel B: Balloon-injured segment demonstrating complete endothelial denudation and deposition of platelet thrombi.

**Figure 3.** Photomicrographs of hematoxylin and eosin sections of arterial segments showing uninjured control (panel A) and balloon-injured (panel B) arterial segments before perfusion with blood. Control segment demonstrates a preserved monolayer of endothelial cells (arrow). Balloon-injured segment reveals endothelial denudation without tears into subintima or media.
by platelet aggregation studies performed on platelet-rich plasma from blood after perfusion through the ex vivo model (Figure 6). When added to citrated platelet-rich plasma (final concentration, 3.3 units/ml), heparin totally inhibited thrombin-induced platelet aggregation (data not shown).

**Effects of PPACK and 7E3 on Platelet Deposition at Site of Balloon Injury**

To evaluate the relative roles of thrombin and GPIIb/IIIa on platelet thrombus formation, the effects of PPACK and 7E3 were studied in our model. Blood treated with heparin alone (2 units/ml), PPACK (10⁻⁵ M), or heparin and murine monoclonal anti-GPIIb/IIIa antibody 7E3 (10 µg/ml) was perfused through the ex vivo system.

With heparin anticoagulation, 8.2±2.2×10⁶ platelets/cm² were deposited at the site of balloon injury compared with 0.7±0.2×10⁶ platelets/cm² on uninjured segments (p<0.02, n=7). Platelet deposition at the site of balloon injury was reduced by 47% with PPACK (10 µM) (n=4, p<0.04) and 70% with 7E3 (10 µg/ml) (n=3, p<0.02) compared with heparin (Figure 7).

Platelet deposition with blood treated with PPACK (10⁻⁵ M) alone was compared with blood treated with PPACK (10⁻⁵ M) and 7E3 (10 µg/ml). Results are normalized to platelet deposition with PPACK-treated blood as 100% deposition (6.2±0.5×10⁶ platelets/cm²). Treatment with 7E3 in addition to PPACK reduced platelet deposition by 56% (p<0.01), suggesting that 44% of thrombin-independent platelet deposition is GPIIb/IIIa independent (Figure 8).

To evaluate the effect of heparin in the presence of total thrombin inhibition by PPACK on platelet deposition, blood was treated with PPACK (10 µM) and heparin (2 units/ml) or PPACK (10 µM) alone. There was no difference in platelet deposition between blood treated with PPACK alone compared with blood treated with PPACK and heparin (n=4, p=0.66).

**Discussion**

The results from these studies demonstrate that at shear rates seen in nonstenotic epicardial coronary arteries, PPACK and 7E3 are more effective than heparin in reducing platelet deposition at the site of balloon injury. Platelet deposition was reduced 47% by PPACK and 70% by 7E3 compared with heparin.

Rabbit thoracic aorta was chosen as the study vessel in our model system because it is well established in the study of human platelet–vessel wall interactions. Weiss et al.²⁸ perfused human blood over everted segments of rabbit aorta to characterize the thrombasthenias and von Willebrand's disease. No differences were observed between rabbit and human platelet depositions on rabbit subendothelium in the Baumgartner and Haudenschild model.⁷ In our system, autologous labeled rabbit platelets were not used because they are not as well characterized as human platelets. Furthermore, the use of rabbit platelets would impose limitations in the evaluation of certain reagents such as monoclonal antibodies, which may specifically recognize human but not rabbit surface proteins.

The ex vivo whole artery model requires the use of an anticoagulant. Heparin was chosen because its actions are well characterized and it is routinely used during angioplasty procedures. The effect of heparin on platelet deposition has been studied using a porcine carotid angioplasty model. In these studies, platelet deposition at the site of superficial injury was not significantly inhibited by a wide range of heparin doses (0.7–14 units/ml plasma). In another study using the same model, there was no difference in platelet deposition in superficially injured arterial segments between animals treated with heparin (50 units/kg/hr) and those that did not receive heparin (control). Given the need for anticoagulation in an ex vivo model, it is not possible to measure platelet deposition in the absence of heparin or other anticoagulants. However, the studies described above, which demonstrate that heparin has little effect on platelet thrombus formation in superficially injured arterial segments, suggest that the platelet deposition measured in our system (with heparin) may reasonably reflect what occurs in nonanticoagulated blood in vivo.

Our results show that thrombin inhibition with PPACK is more effective than heparin-ATIII in reducing platelet deposition at the site of balloon injury (Figure 7). The increased potency of thrombin inhibition with PPACK compared with heparin is consistent with studies using a baboon arteriovenous fistula model in which platelet deposition on Dacron graft material is measured.⁶ Although both PPACK and heparin-ATIII work via the same mechanism to inhibit thrombin (i.e., binding to the serine protease active site), there are several important differences
FIGURE 5. Platelet aggregation curve demonstrating effect of d-Phe-Pro-ArgCH₂Cl (PPACK) (P) on thrombin (T)- or collagen (C)-induced platelet aggregation. PPACK (10⁻⁵ M) T (1 unit/ml), and collagen (5 μg/ml) were sequentially added to citrated platelet-rich plasma. PPACK inhibited thrombin-induced platelet activation but had no effect on subsequent collagen-induced aggregation.

FIGURE 6. Platelet aggregation curves demonstrating effect of 7E3 on collagen (C)-induced aggregation. Heparinized whole blood with or without 7E3 (10 μg/ml) was perfused through ex vivo system. After perfusion, platelet-rich plasma was prepared. 7E3 completely inhibited collagen-induced (5 μg/ml) aggregation.
between these two agents that may explain the greater antiplatelet effects observed with PPACK.6,31,32 Platelet factor 4, released by activated platelets at the site of vascular injury, neutralizes heparin but is not known to inhibit the action of PPACK.33 In addition, PPACK may have greater access to thrombin because it is smaller than the heparin-ATIII complex. The PPACK-thrombin complex may also bind to the platelet thrombin receptor without platelet activation and therefore competitively inhibit activation by free thrombin.4 Finally, PPACK inhibits meizothrombin, whereas heparin-ATIII does not.34 Meizothrombin, an intermediate in α-thrombin generation, is dependent on the membrane-bound prothrombinase complex. When membrane bound, meizothrombin is a fully active serine protease and resistant to inactivation by ATIII-heparin complex.35

Platelet thrombus formation is a complex process involving multiple pathways of activation leading to platelet adhesion and aggregation. Analysis of the PPACK and 7E3 data may provide insights into this process. The platelet membrane GPIIb/IIIa is the surface receptor for fibrinogen as well as other adhesive proteins such as von Willebrand’s factor and fibronectin.12-14 GPIIb/IIIa antagonists have been shown to decrease platelet deposition on collagen strips and Dacron vascular grafts and prevent coronary reclosure during thrombolysis.16,36-38 The 70% reduction in platelet deposition by 7E3 may be viewed operationally as representing the inhibition of GPIIb/IIIa-mediated platelet-platelet cohesion or aggregation.

The observation that PPACK reduced platelet deposition by 47% compared with heparin suggests that the majority (47 of 70, or approximately two thirds) of GPIIb/IIIa platelet-platelet cohesion is secondary to heparin-resistant thrombin stimulation. Our observation that heparin had no additive effect on platelet deposition when added to PPACK suggests that heparin does not inhibit platelet deposition by mechanisms other than thrombin inhibition. From these observations and the previously mentioned in vivo studies, we conclude that the substantial reduction in platelet deposition by PPACK compared with heparin may reasonably reflect what occurs in vivo in the absence of an anticoagulant and establishes the importance of heparin-resistant thrombin in platelet thrombus formation.

The further reduction in platelet deposition by 7E3 in the presence of PPACK (Figure 8) demonstrates the existence of thrombin-independent pathways of platelet activation. This may be the result of ADP, serotonin, platelet-activating factor, or other agonists that are released or exposed at the site of vascular injury.

Platelet deposition in the presence of 7E3, which accounts for approximately 30% of the total (Figure 7), presumably is not related to platelet-platelet cohesion and most likely represents non-GPIIb/IIIa-mediated platelet adhesion to the exposed matrix proteins (e.g., collagen, von Willebrand’s factor, fibronectin, or laminin). Recently, specific platelet receptors for collagen, fibronectin, and von Willebrand’s factor have been identified as GPIa-IIa, GPIc-IIa, and GPIb-IX, respectively.14,39-41 The roles of these adhesion molecules can
be tested in our model system using monoclonal antibodies or synthetic peptides.

These data demonstrate the importance of both thrombin and GPIIb/IIIa in platelet thrombus formation in non-diseased, superficially injured elastic arteries. It is important to recognize that the injury induced by our protocol is limited to endothelial denudation with minimal medial disruption. Deep injury may occur when angioplasty is performed in muscular or diseased arteries. Using an in vivo porcine model, Steele et al reported greater platelet deposition when deep injury is induced. The relative importance of thrombin and GPIIb/IIIa may vary with deep injury, shear rates, and atherosclerotic changes within the subendothelial matrix.

The effect of thrombin and GPIIb/IIIa inhibition as analyzed in our model may have important implications when designing regimens to reduce early reocclusion and late restenosis after angioplasty. When contemplating the clinical use of GPIIb/IIIa and thrombin antagonists, the potential for major hemorrhagic complications is of concern. In a recent study, unstable angina patients received large enough doses of 7E3 to inhibit platelet aggregation and markedly prolong bleeding time without major bleeding complications. With thrombin inhibition, impairment of platelet function may be less severe, as demonstrated in this study. On the other hand, there is also inhibition of fibrin formation with the use of thrombin inhibitors, which may increase the risk of bleeding. Further study of thrombin and GPIIb/IIIa inhibitors alone or in combination will be required to assess the clinical efficacy and safety of these agents.

Acknowledgments

We wish to thank Nora Gurevich and the staff of the Division of Nuclear Medicine for their help with platelet labeling and Chris Swanson and Baz Hudal, MD, for their technical assistance. We would also like to thank Barry S. Coller, MD, for his generous gift of 7E3.

References


KEY WORDS • thrombus • aggregation • arterial injury
Roles of thrombin and platelet membrane glycoprotein IIb/IIIa in platelet-subendothelial deposition after angioplasty in an ex vivo whole artery model.
A V Kaplan, L L Leung, W H Leung, G W Grant, I R McDougall and T A Fischell

Circulation. 1991;84:1279-1288
doi: 10.1161/01.CIR.84.3.1279

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/84/3/1279

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/