

Activation of Platelets in Platelet-Rich Plasma by Rotablation Is Speed-Dependent and Can Be Inhibited by Abciximab (c7E3 Fab; ReoPro)

Marlene S. Williams, MD; Barry S. Collier, MD; Heikki J. Väänänen, MSc; Lesley E. Scudder, BSc; Samin K. Sharma, MD; Jonathan D. Marmur, MD

Background—Rotational atherectomy with the Rotablator catheter has improved percutaneous treatment of certain coronary atherosclerotic lesions, but the “no-reflow” phenomenon remains a serious complication. Because platelet activation by rotablation may contribute to the no-reflow phenomenon, we developed an in vitro system to test the effect of rotablation on platelets in the absence or presence of platelet GP IIb/IIIa receptor blockade with abciximab.

Methods and Results—Platelet-rich plasma (PRP) was prepared from 28 healthy human volunteers. PRP was divided into 4 samples: (1) no treatment, (2) 6D1 (anti-GP Ib), (3) c7E3 Fab (anti-GP IIb/IIIa+ $\alpha_v\beta_3$), and (4) c7E3 Fab+6D1. Samples were pumped through a flow chamber containing a 2.5-mm burr rotating at various speeds and then placed in an aggregometer. PRP samples tested in the absence of antibody underwent more rapid and extensive aggregation when rotablated at 150 000 and 180 000 rpm compared with 0 rpm ($P<0.001$ at both speeds). Preincubation of platelets with c7E3 Fab decreased the slope of aggregation at each rotablation speed, with 98%, 79%, and 71% reductions at 70 000, 150 000, and 180 000 rpm, respectively ($P=0.09$ for 70 000 and $P<0.001$ for both 150 000 and 180 000 rpm). Preincubation of platelets with 6D1 did not decrease the slope of aggregation at any rotablation speed ($P>0.5$, $P=0.99$, and $P=0.091$ for 70 000, 150 000, and 180 000 rpm). Platelet ATP release, a marker of granule release and cell damage, was markedly increased at 180 000 rpm ($P=0.002$ compared with 0 rpm in the control group). Electron microscopy revealed extensive rotablation-induced platelet damage at 150 000 and 180 000 rpm, and leakage of LDH confirmed platelet lysis at these speeds ($P=0.002$ and $P<0.001$ compared with 0 rpm).

Conclusions—High-speed rotablation induces platelet activation of PRP, leading to aggregation; pretreating PRP with abciximab decreases the aggregation. These data suggest that pretreatment of patients with abciximab may decrease rotablation-induced platelet aggregation during rotational atherectomy. (*Circulation*. 1998;98:742-748.)

Key Words: platelets ■ platelet aggregation inhibitors ■ angioplasty

Rotational atherectomy with the Rotablator device (Boston Scientific Corp) has improved percutaneous treatment of complex coronary atherosclerotic lesions, including long lesions with irregular borders, lesions with heavy calcification, and lesions at ostial locations.¹⁻³ The Rotablator device consists of a nickel-plated brass elliptical burr whose distal end is coated with diamond microchips, connected by a flexible Teflon-sheathed drive shaft to a gas-driven turbine that rotates at speeds up to $\approx 180\,000$ rpm.⁴ The burrs come in variable diameters ranging from 1.25 to 2.5 mm. The rotating burr is designed to pulverize inelastic plaque without damaging healthy elastic tissue, leaving behind a smooth surface.^{5,6} A serious complication of rotational atherectomy is the “no-reflow” phenomenon, which is characterized by reduced or no blood flow through the treated vessel despite successful diminution in the proximal stenosis. Distal embol-

ization of pulverized plaque, coronary spasm, and platelet activation all may contribute to the pathogenesis of the no-reflow phenomenon. On the basis of our anecdotal observation that no-reflow rarely occurs in patients pretreated with abciximab (c7E3 Fab, ReoPro), which blocks platelet GP IIb/IIIa receptors and inhibits platelet aggregation, we hypothesized that the Rotablator device may activate platelets, leading to platelet aggregation and thrombus formation. We further hypothesized that the GP IIb/IIIa receptor may play a significant role in this aggregation response.

To test these hypotheses, we designed an in vitro system to simulate the in vivo effects of the Rotablator and studied the effects of the Rotablator on PRP in the absence and presence of abciximab. In addition, because shear forces near the rotating burr may activate platelets and because platelet GP Ib has been implicated in mediating platelet activation induced

Received November 18, 1997; revision received March 16, 1998; accepted April 17, 1998.

From the Divisions of Cardiology and Hematology, Department of Medicine, Mount Sinai School of Medicine, New York, NY.

Dr Collier is an inventor of abciximab and, in compliance with federal law and the patent policy of the Research Foundation of the State University of New York, shares in the royalties paid to the Foundation from the sale of abciximab.

Correspondence to Jonathan D. Marmur, MD, The Mount Sinai Medical Center, Division of Cardiology, Box 1030, One Gustave L. Levy Place, New York, NY 10029. E-mail jmarmur@smtplink.mssm.edu

© 1998 American Heart Association, Inc.

Selected Abbreviations and Acronyms

GP = glycoprotein
LDH = lactate dehydrogenase
PPP = platelet-poor plasma
PRP = platelet-rich plasma

by shear forces,^{7,8} we also studied the effect of a well-characterized antibody to GP Ib (6D1) in the system.

Methods

Platelet Preparation

After informed consent was obtained from 28 healthy human donors (19 male, 9 female) between the ages of 23 and 59 years who had not taken any medication known to inhibit platelet function for at least 7 days, peripheral venous blood (60 mL) was collected via a 19-gauge butterfly needle into 2 tubes each containing 0.3 mL of 40% sodium citrate solution. PRP was prepared by centrifugation at 700*g* for 3.5 minutes at 22°C and slowly removed with a plastic pipette. PPP was prepared by further centrifugation of the remaining blood at 3000*g* for 10 minutes at 22°C.

Platelet counts were determined with an automated resistive counter (Coulter model Z1), and the platelet count was adjusted to $\approx 300\,000/\mu\text{L}$ with PPP. Actual final platelet counts ranged from 279 000 to 464 000/ μL , with a mean of $317\,800 \pm 43\,300/\mu\text{L}$.

Antibody Treatment

PRP was pretreated with 20 $\mu\text{g}/\text{mL}$ of c7E3 Fab (anti-GP IIb/IIIa + $\alpha_2\beta_1$; Centocor; stock solution, 1 mg/mL in 0.01 mol/L phosphate, 0.15 mol/L NaCl, 0.0005% Tween 80; lot 92H08AA)⁹ or with 20 $\mu\text{g}/\text{mL}$ of 6D1 (anti-GP Ib), prepared as previously described¹⁰ (stock solution, 1 mg/mL in 0.15 mol/L NaCl, 0.01 mol/L Tris/HCl, 0.05% azide, pH 7.4) for at least 20 minutes at 22°C. For the samples with combined c7E3 Fab and 6D1, PRP was first pretreated with 20 $\mu\text{g}/\text{mL}$ of 6D1 for 20 minutes and then further treated with 20 $\mu\text{g}/\text{mL}$ of c7E3 Fab for at least 20 minutes at 22°C. For c7E3 Fab dose-response studies, PRP was incubated with c7E3 Fab at 1, 2, 5, 10, 20, 50, or 100 $\mu\text{g}/\text{mL}$. A total of 10 different donors were used for this experiment.

Acetylsalicylic Acid (Aspirin) Treatment

PRP was pretreated with 100 $\mu\text{mol}/\text{L}$ aspirin (Mallinckrodt, lot 2004KJLL; stock solution, 5 mmol/L in 0.01 mol/L phosphate, 0.15 mol/L NaCl, pH 7.0) for at least 5 minutes at 22°C. For the samples pretreated with both aspirin and c7E3 Fab, PRP was first pretreated with 20 $\mu\text{g}/\text{mL}$ of c7E3 Fab as described above and then further pretreated with aspirin. To ensure that the donor had not taken aspirin and that the *in vitro* aspirin treatment eliminated thromboxane A_2 production, arachidonic acid (0.8 mmol/L)-induced aggregation was tested before and after aspirin incubation in each experiment. In each case, the platelets aggregated briskly in response to arachidonic acid before aspirin treatment and did not respond at all after aspirin treatment. This experiment was repeated with the blood from 5 different donors.

Rotablation Model

PRP (5 to 6 mL) was placed into a plastic 20-mL syringe. Another 20-mL syringe was filled with an equal volume of degassed normal saline. Both syringes were attached to the same infusion pump (Harvard model 975), which was set to run at 4 mL/min. A 3 $\times$ 21-mm acrylic chamber (Figures 1 and 2) designed to house a 2.5-mm Rotablator burr, with inlet and outlet ports, was constructed to immobilize the guidewire and limit the lateral and back-and-forth movements of the burr. The PRP was pumped into the inlet port, and the saline was pumped into the chamber via the Rotablator sleeve and the gap between the guidewire and the center hole in the burr. The mixture of saline and PRP exited the chamber via the outlet port.

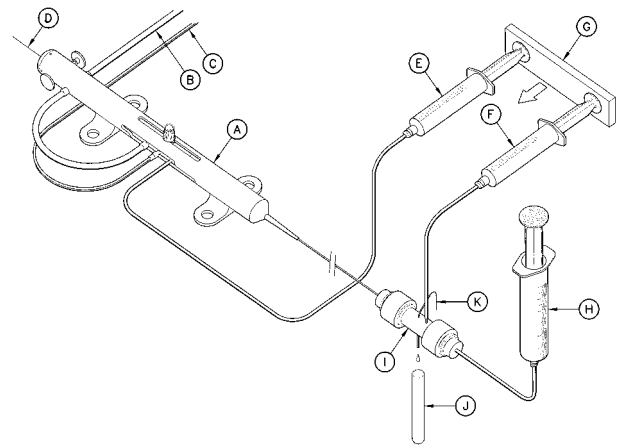


Figure 1. Flow chamber design with Rotablator connections detailed above. A, Rotablator housing; B, compressed gas (turbine) connector; C, fiberoptic tachometer cable; D, guidewire; E, syringe containing saline connected to saline infusion port (20 mL); F, syringe (20 mL) containing PRP; G, Harvard pump; H, syringe containing saline used to create an air-free "saline seal"; I, acrylic flow chamber; J, 1.5-mL microfuge tube; K, thermocouple. PRP flows from F→I→J.

The temperature within the chamber (measured and recorded before and after each run) was monitored with a thermocouple (Sensortek, model BAT-12) located distal to the burr. The temperature did not change significantly when the speed of rotablation was 70 000 rpm, increased by $\approx 1^\circ\text{C}$ to 2°C when the speed was 150 000 rpm, and increased by $\approx 2^\circ\text{C}$ when the speed was 180 000 rpm. Before the Rotablator was turned on, PRP and saline were pumped into the chamber and visible air bubbles were expressed from the PRP-saline mixture. To avoid air entry into the chamber during rotablation, a fluid seal was created around the protruding portion of the guidewire with tubing attached to a syringe filled with saline. To obtain baseline control platelet samples, PRP and saline were pumped through the chamber for 5 seconds with the burr stationary. The Rotablator was then run for 5 seconds at $70\,000 \pm 5000$, $150\,000 \pm 5000$, or $180\,000 \pm 5000$ rpm while PRP and saline were pumped through the chamber. The PRP/saline samples exiting from the chamber were immediately tested for aggregation, microscopy, LDH release, recalcification time, or luminescence as described below. On the basis of the chamber volume (148 μL) and the pump

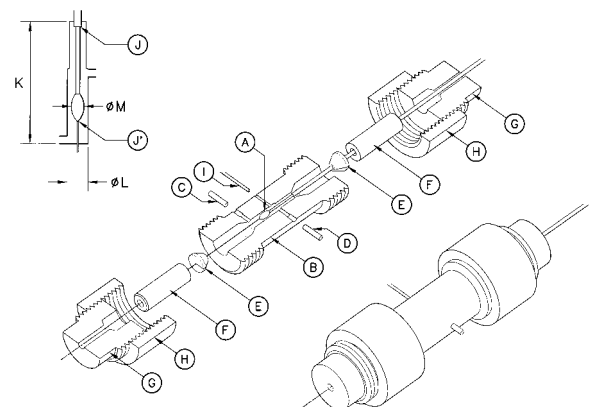


Figure 2. Detailed diagram of flow chamber. A, 2.5-mm burr; B, chamber body; C, inlet port; D, outlet port; E, gaskets; F, gasket plugs; G, end piece; H, lock ring; I, thermocouple. Inset: J, saline access into flow chamber from Rotablator sleeve; J', saline access into flow chamber from guidewire hole in burr; K, length of chamber (21 mm); ϕL , diameter of chamber (3 mm); ϕM , diameter of burr (2.5 mm).

rate (4 mL/min for both the PRP and saline), a platelet passed through the chamber in 1.1 seconds. The pH of each PRP sample was measured before and immediately after rotablation, and the results were not significantly different.

Platelet Aggregation

Effluent PRP (0.4 mL) was immediately added to a siliconized aggregometer cuvette containing a 2×4-mm cylindrical, Teflon-coated stir bar, and then the cuvette was placed in an aggregometer well preheated to 37°C (Chrono-Log model 530). PPP was used as the blank. The aggregation response, measured as the initial slope,¹¹ was assessed for ≈5 minutes. To ensure that the platelets in the PRP were able to respond to a well-defined agonist, the baseline PRP was treated with 5 μmol/L ADP and the change in light absorbance was measured for ≈5 minutes. This experiment was repeated 10 times with blood from 10 different donors.

Microscopy

PRP samples (100 μL) were removed from the aggregometer cuvette after ≈5 minutes of stirring and fixed with 33 μL of 4% paraformaldehyde for a final concentration of 1% for 5 minutes at 22°C. Then 20 μL of each sample was placed on a glass microscope slide, covered with a coverslip, and viewed by differential interference and phase-contrast microscopy (Olympus System, model BX60). Photographs were taken with ASA 100 film (Kodak 100). For transmission electron microscopy, PRP was fixed with paraformaldehyde (final concentration, 1%) for 5 minutes at 22°C and then centrifuged at 3000g for 6 minutes at 22°C. The resulting pellet was then fixed with 3% glutaraldehyde in 0.2 mol/L sodium cacodylate buffer at pH 7.4 for 3 hours, washed in PBS buffer, treated for 1 hour with 1% osmium tetroxide, dehydrated in graded steps of ethanol through propylene oxide, and embedded in epoxy resin (Embed 812; Epon 812). Representative areas for ultrathin sections were chosen by light microscopy from 1-μm plastic sections stained with methylene blue and azure II. Ultrathin sections were stained with uranyl acetate and lead citrate. The platelets were observed with a JEM 100CX transmission electron microscope. Electron microscopy was repeated 2 times with blood from 2 different donors.

Luminescence Studies

Effluent PRP (450 μL) and luminescence reagent (50 μL Chrono-Lume, Chrono-log) were placed into a siliconized cuvette, and then luminescence due to ATP release from platelets and platelet aggregation were measured simultaneously in the aggregometer. ATP release was quantified by addition of 2 nmol of ATP to the sample as an internal control at the end of the experiment. Luminescence of untreated PRP was also measured after activation with 1 U/mL of human thrombin (Sigma Chemical Co). This experiment was repeated 5 times with blood from 5 different donors.

LDH Assay

After rotablation of PRP at the various speeds, PPP was prepared from the PRP samples as above, and plasma LDH was measured by the Technicon Omnipak LDH method (Technicon Instruments Corp).¹² To determine maximum LDH release, PRP was subjected to 4 rounds of freezing and thawing; the PRP was then centrifuged and the supernatant tested. This experiment was repeated 3 times with blood from 3 different donors.

Clotting Assay

Effluent PRP (200 μL) before and after rotablation at 180 000 rpm was placed into a glass borosilicate tube (12×75 mm) and placed in a 37°C water bath for 15 seconds. Calcium chloride (0.1 mL of 0.025 mol/L) was added to the PRP, a stopwatch was started, and the tube was inverted every 30 seconds to test for the onset of clot formation. This clotting assay was repeated for PPP, as well as PRP frozen and thawed 4 times and PRP aggregated with 5 μmol/L ADP. To assess any temporal drift in clotting times during an experiment, control PRP samples were tested at intervals throughout the experiment. This experiment was repeated with blood from 3 different donors.

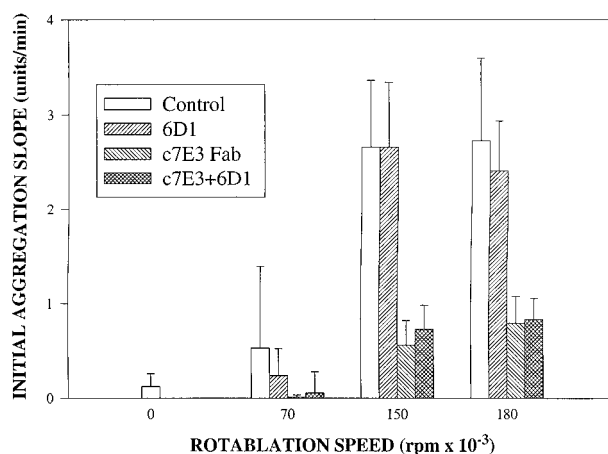


Figure 3. Effect of rotablation on platelet aggregation and effects of antibodies 6D1 and c7E3 Fab. Platelet aggregation results expressed as slope (mean+SD) in transmission light units/min plotted against Rotablator speed. $P<0.001$ for the c7E3 Fab and c7E3+6D1 groups compared with control (no treatment) groups. $N=10$.

Statistics

Data were analyzed by 3-way ANOVA (patient, speed, and aspirin and/or antibody treatment), and once significance was determined, further randomized block ANOVA was performed within each speed. When the initial analysis was not significant, the results are reported as $P=NS$. Further analysis for differences in speed were performed for the control group. Pairwise multiple comparisons were performed with the Bonferroni adjustment.

Results

Effect of Rotablation on Platelet Aggregation

When PRP was pumped through the chamber with the Rotablator burr stationary and then placed into an aggregometer cuvette and stirred at 1200 rpm at 37°C, essentially no platelet aggregation was observed (Figure 3). The slope of platelet aggregation increased slightly when the PRP was exposed to rotablation at 70 000 rpm, but the difference was not significant ($P=0.16$). In contrast, aggregation increased dramatically when the speed of rotablation was increased to 150 000 rpm ($P<0.001$ compared with 0 rpm); there was little further increase in the aggregation slope when the speed was increased to 180 000 rpm ($P<0.001$ compared with 0 rpm; $P=0.8$ compared with 150 000 rpm).

Effect of Antibodies 6D1 and c7E3 Fab on Rotablation-Induced Platelet Aggregation

Preincubation of the PRP with 20 μg/mL of antibody 6D1 had little effect on the slope of aggregation (Figure 3). In contrast, preincubation of platelets with 20 μg/mL c7E3 Fab consistently decreased the slope of aggregation at each rotablation speed, with 98%, 79%, and 71% reductions at 70 000, 150 000, and 180 000 rpm, respectively ($P=0.09$ for 70 000 and <0.001 for both 150 000 and 180 000 rpm). Preincubating PRP with both 6D1 and c7E3 Fab did not result in greater inhibition of aggregation than was observed with c7E3 Fab alone ($P=0.32$ at 150 000 rpm and $P=NS$ at 180 000 rpm). c7E3 Fab dose-response studies demonstrated maximal inhibition of platelet aggregation at ≈10 μg/mL,

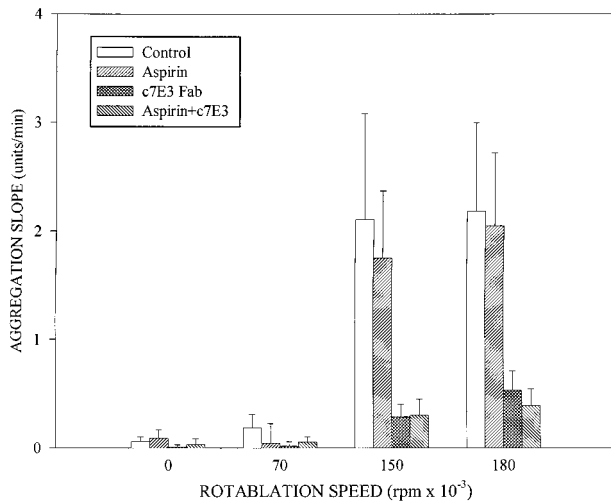


Figure 4. Effect of aspirin and c7E3 Fab on rotablation-induced platelet aggregation. Platelet aggregation results expressed as slope (mean $\pm$ SD) in transmission light units/min plotted against Rotablator speed. $N=5$.

with no additional inhibition even at concentrations of 100 $\mu\text{g/mL}$ (data not shown).

Effect of Aspirin on Rotablation-Induced Platelet Aggregation

Preincubating PRP with aspirin reduced the slope of aggregation induced by 70 000 rpm by 79% ($P=0.1$) (Figure 4) but reduced the slopes of aggregation at 150 000 and 180 000 rpm by only 17% and 6% ($P=0.46$ and 0.80 compared with control at 150 000 and 180 000 rpm, respectively). Addition of aspirin to c7E3 Fab-treated samples did not significantly increase the inhibition of platelet aggregation ($P=\text{NS}$, 0.99, and 0.57 for 70 000, 150 000, and 180 000 rpm).

ATP Release in Response to Rotablation

Platelet ATP release, measured as luminescence, increased with increasing rotablation speed ($P=0.99$ for 0 versus 70 000 rpm), with increases at 150 000 ($P=0.18$) and 180 000 ($P=0.002$) rpm (Figure 5). Maximal luminescence was observed immediately after the PRP sample was added to

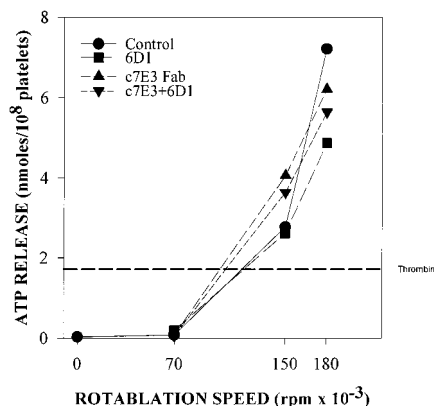


Figure 5. Mean ATP release (nmol ATP/ 10^8 platelets) in response to rotablation at different speeds. For comparison, release produced by 1 U/mL thrombin in the absence of rotablation (1.72 nmol/ 10^8 platelets) is shown by dashed line. $N=5$.

Clotting of PRP After Recalcification

Sample	Time, s
Control	149 $\pm$ 23
Rotablation	90 $\pm$ 0
Rotablation+aspirin	86 $\pm$ 8
Rotablation+c7E3 Fab	90 $\pm$ 0
Rotablation+aspirin+c7E3 Fab	98 $\pm$ 15
F/T	75 $\pm$ 17
ADP	102 $\pm$ 15
PPP	260 $\pm$ 42

Clotting of PRP after recalcification. PRP was exposed to rotablation at 180 000 rpm except in the control group. For comparison of clot formation times, PRP was frozen and thawed (F/T) or treated with ADP. The clotting time of PPP was also measured. Values are mean $\pm$ SD.

the luminescence aggregometer, and then the luminescence decreased. ATP release produced by exposure to rotablation at 150 000 and 180 000 rpm exceeded release induced by 1 U/mL of thrombin, suggesting that release might be coming from cytoplasmic as well as granular stores of ATP. c7E3 Fab and/or 6D1 did not inhibit ATP release.

Platelet Morphological Changes Produced by Rotablation

When assessed by phase-contrast microscopy, platelets that were pumped through the chamber with the Rotablator burr stationary and then stirred in an aggregometer cuvette were discoid, with few spikelike filopodia (Figure 6, top). Platelets exposed to rotablation at 180 000 rpm and then stirred in the aggregometer for 5 minutes exhibited extensive platelet aggregation, and individual platelets showed spikelike filopodia (Figure 6, bottom). When assessed by transmission electron microscopy (Figure 7, top), platelets in PRP that was not exposed to rotablation had an intact membrane and evenly dispersed granules. They were disk-shaped or rounded, with relatively few filopodia. Fewer than 1% had a ghostlike appearance. In contrast, 90% of platelets exposed to rotablation at 180 000 rpm had ruptured membranes and/or a ghostlike appearance, with loss of granules and the assumption of a more rounded shape (Figure 7, bottom). In addition, the background was very grainy, suggesting the possibility of plasma and/or platelet protein precipitation.

Lactate Dehydrogenase

Platelets exposed to rotablation demonstrated significant speed-dependent release of the intracellular enzyme LDH (Figure 8). For untreated PRP, $P=0.85$ for 0 versus 70 000 rpm, $P=0.002$ for 0 versus 150 000 rpm, and $P<0.001$ for 0 versus 180 000 rpm. At 180 000 rpm, this release was not significantly affected by pretreatment with 6D1, c7E3 Fab, or a combination of the two. The release of LDH due to platelet lysis from repeated freezing and thawing, however, far exceeded the release caused by rotablation at 180 000 rpm.

Clotting Time

PPP had a recalcification clotting time of 260 $\pm$ 42 seconds, whereas PRP had a recalcification clotting time of 149 $\pm$ 23 seconds, ADP-treated PRP 102 $\pm$ 15 seconds, frozen and

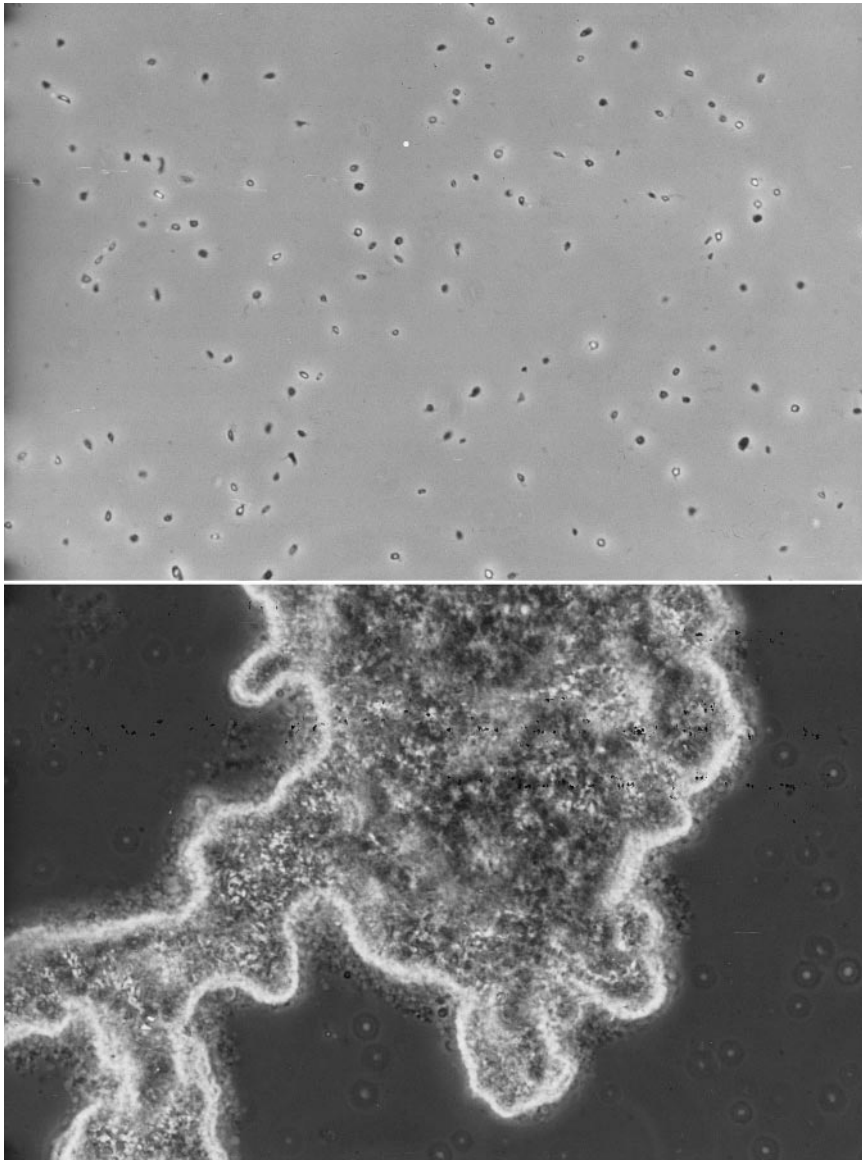


Figure 6. Phase-contrast microscopy at $\times 400$ magnification. Top, Platelets passed through flow chamber with rotablation burr stationary. Bottom, Platelets passed through flow chamber with rotablation at 180 000 rpm with a total exposure time of 1.1 seconds, resulting in a large platelet aggregate.

thawed PRP 75 ± 17 seconds, and PRP exposed to rotablation at 180 000 rpm 90 ± 0 seconds (Table). These data indicate that platelets can facilitate clot formation in this system and that maximal platelet support of coagulation (as shown by the frozen and thawed sample) can only reduce the clotting time to ≈ 75 seconds. Exposure of PRP to rotablation at 180 000 rpm significantly shortened the recalcification clotting time ($P=0.02$ compared with the nonrotablated sample) but did not shorten the clotting time to the same extent as freezing and thawing. Antibody c7E3 Fab or aspirin pretreatments did not prevent the shortening of the clotting times.

Discussion

Our data indicate that exposure of platelets in PRP to rotablation at 150 000 rpm or more for as little as 1.1 seconds can cause the platelets to be activated such that they undergo aggregation when stirred at 37°C . Although our experimental procedures differ significantly, our data are consistent with the preliminary report by Reisman et al¹³ of speed-dependent, rotablation-induced platelet aggregation of heparinized

miniswine whole blood. We also observed in our PRP system that c7E3 Fab significantly inhibits but does not eliminate rotablation-induced platelet aggregation. In contrast, antibody 6D1 had minimal effects on aggregation, and adding 6D1 to c7E3 Fab did not enhance the c7E3 Fab effect. Although aspirin appeared to have a small effect in decreasing platelet aggregation in response to low-speed rotablation, it was not statistically significant. Combining aspirin with c7E3 Fab did not further reduce platelet aggregation. Thus, thromboxane A_2 production does not seem to be crucial for high-speed rotablation-induced platelet aggregation, whereas much but not all of the aggregation depends on the GP IIb/IIIa receptor.

High shear stress produced by a cone-and-plate viscometer or other devices has been shown to activate platelets and lead to platelet aggregation.^{14,15} Previous work suggests that the mechanism of activation involves the binding of von Willebrand factor to GP Ib, followed by an increase in cytoplasmic ionized calcium, leading to activation of the GP IIb/IIIa receptor, binding of fibrinogen to GP IIb/IIIa, and finally aggregation of platelets.¹⁶ Shear-induced platelet acti-

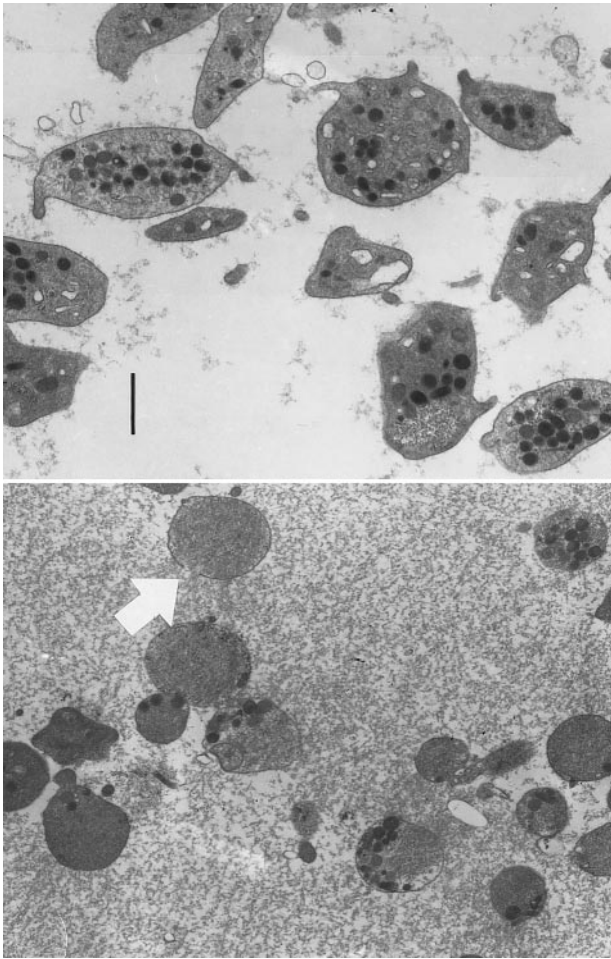


Figure 7. Transmission electron micrography. Top, PRP pumped through chamber with Rotablator burr held stationary (0 rpm) and stirred in an aggregometer for 5 minutes. Note presence of intact platelet membrane, intracellular granules, and clear background. Bar=1.2 μm . Bottom, PRP was subjected to rotablation at 180 000 rpm and then stirred in an aggregometer for 5 minutes. Observe ruptured platelet membrane (arrow), depletion of intracellular organelles (ghost platelets), and cloudy background.

vation and aggregation has consistently been inhibited in other model systems by blocking of the GP Ib receptor,^{7,8,17} and yet the 6D1 antibody was not effective in our studies. This suggests that the mechanism of rotablation-induced platelet activation differs from that induced by high shear forces. Because we observed release of ATP when platelets were exposed to rotablation speeds of 150 000 and 180 000 rpm, it is possible that the aggregation is largely due to a combination of activation-dependent and lytic release of ADP from dense granules and the cytoplasmic pool of adenine nucleotides at the same time that the ATP is released from the same sites. Our results with c7E3 Fab are consistent with this interpretation, because c7E3 Fab blocks ADP-induced platelet aggregation.⁹ Because c7E3 Fab did not completely eliminate platelet aggregation, other mechanisms may be involved.

Electron microscopy and LDH measurements provided further evidence of rotablation-induced platelet lysis. Transmission electron micrographs of PRP subjected to rotablation

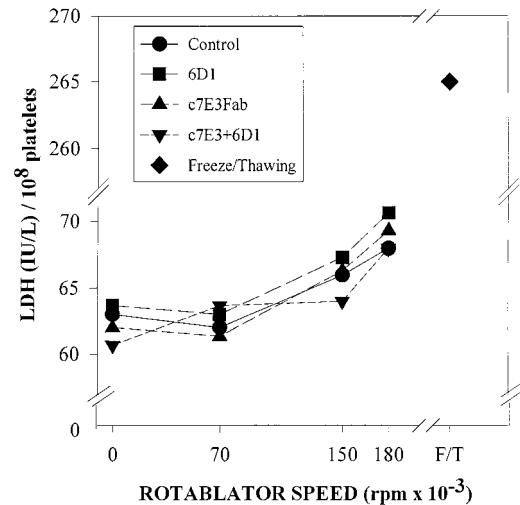


Figure 8. Supernatant LDH after PRP was exposed to rotablation or freezing and thawing (F/T).

at 180 000 rpm demonstrated rupture of plasma membranes, loss of intact granules, and the appearance of rounded forms. High-speed rotablation also led to LDH release, providing additional evidence of platelet lysis. The lysis, however, was considerably less than that caused by repeated freezing and thawing.

Plasma coagulation consists of a series of enzymatic reactions culminating in the conversion of prothrombin to thrombin, which converts plasma fibrinogen to insoluble fibrin. In vitro coagulation may be initiated via either the intrinsic or extrinsic pathways.¹⁸ Several coagulation reactions are dramatically accelerated by the presence of phospholipids or platelet membranes. Unactivated platelets are less active in supporting thrombin generation than activated or lysed platelets, because activation or lysis increases the number of negatively charged phospholipid molecules on the platelet surface.¹⁹ Activation may also lead to prothrombin binding to GP IIb/IIIa and facilitation of prothrombin conversion to thrombin.²⁰ The decrease in clotting time we observed in PRP subjected to rotablation provides additional support for our interpretation that rotablation causes platelet activation and lysis. Moreover, it suggests that in addition to causing platelet aggregation, rotablation may facilitate thrombin generation and clot formation as additional mechanisms by which it may induce the no-reflow phenomenon. Previous studies on the effect of c7E3 Fab on thrombin generation in gel-filtered platelets demonstrated that c7E3 Fab was able to inhibit $\approx 47\%$ of tissue factor-induced thrombin generation¹⁹; however, when platelets were stimulated with calcium ionophore A23187, a potent direct platelet activator, the subsequent addition of c7E3 Fab did not significantly reduce thrombin generation. Therefore, the inability of c7E3 Fab to affect the shortening of clotting time induced by rotablation is consistent with either lysis or direct activation of platelets by rotablation.

Our studies have potential implications for the clinical use of the Rotablator device. The speed dependence of platelet activation that we and Reisman et al¹³ have found suggests that it may be desirable to use the lowest speed that is

effective in removing plaque. In addition, the decrease in platelet aggregation produced by c7E3 Fab raises the possibility that pretreatment of patients with abciximab may decrease platelet aggregation during rotational atherectomy. The recent preliminary report of Koch et al²¹ demonstrating a reduction in ischemic myocardial regions during rotablation in patients treated with abciximab compared with matched control subjects supports a potential benefit of abciximab treatment, but clinical trials will be required to confirm these results.

Limitations of This Study

Our in vitro system differs from clinical practice in a number of respects: (1) we used PRP rather than whole blood and thus did not study possible effects of erythrocytes, such as release of ADP in response to cellular injury, that may contribute to platelet aggregation; (2) our studies were conducted at 22°C rather than at 37°C; and (3) it is possible that the Rotablator exposure time for platelets in this system is greater than that in vivo in small coronary arteries, but this will depend on whether the coronary artery is narrower or wider than the chamber (3 mm) and whether blood flow is >8 mL/min during the rotablation procedure. Data indicate that blood flow in a diseased coronary artery is ≈10 mL/min,²² but the flow rate may be altered by the insertion of the Rotablator burr into the blood vessel. Thus, it is difficult to estimate the blood flow during rotablation. This value is needed to estimate the percentage of platelets in the body that will transit past the Rotablator in a single procedure. From studies of 9 patients in our institution, we found that the average time of rotablation was 3±0.5 minutes. Assuming that the Rotablator burr does not affect blood flow, ≈30 mL of blood will be exposed to the Rotablator, containing ≈0.6% of the total circulating platelet volume. The percentage of platelets indirectly affected by rotablation could be much greater, however, because released ADP may activate platelets that did not transit past the Rotablator.

Acknowledgments

This research was supported by the American College of Cardiology/Merck Fellowship Award and in part by grants HL-19278 and HL-54469 from the National Heart, Lung, and Blood Institute. The authors wish to thank Dr Sylvan Wallerstein for his help with the statistical analysis, and Dr Ronald Gordon for performing the electron microscopy. We would also like to thank Heart Technology (Seattle, Wash) for the generous donation of the Rotablator system and the volunteers who donated their blood.

References

- Bowles M, Palko W, Beaver C, Cowley C, Kipperman R. Clinical and postmortem outcome of "no-reflow" phenomenon in a patient treated with rotational atherectomy. *South Med J*. 1996;89:820–823.
- Stertzer SH, Pomerantsev EV, Fitzgerald PJ, Shaw RE, Walton AS, Singer AH, Yeung A, Yock PG, Oesterle SN. Effects of technique modification on immediate results of high speed rotational atherectomy in 710 procedures on 656 patients. *Cathet Cardiovasc Diagn*. 1995;36:304–310.
- Ellis SG, Popma JJ, Buchbinder M, Franco I, Leon MB, Kent KM, Pichard AD, Satler LF, Topol EJ, Whitlow PL. Relation of clinical presentation, stenosis morphology, and operator technique to the procedural results of rotational atherectomy and rotational atherectomy-facilitated angioplasty. *Circulation*. 1994;89:882–892.
- MacIsaac AI, Bass TA, Buchbinder M, Cowley MJ, Leon MB, Warth DC, Whitlow PL. High speed rotational atherectomy: outcome in calcified and noncalcified coronary artery lesions. *J Am Coll Cardiol*. 1995;26:731–736.
- McCarty LH. Catheter clears coronary arteries: diamond-studded Rotablator cleans blocked arteries without damaging walls. *Design News*. 1991;88–91.
- Reisman M, Buchbinder M. Rotational ablation: the Rotablator catheter. *Cardiol Clin*. 1994;12:595–610.
- 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.
- Goto S, Salomon DR, Ikeda Y, Ruggieri ZM. Characterization of the unique mechanisms mediating the shear-dependent binding of soluble von Willebrand factor to platelets. *J Biol Chem*. 1995;270:23352–23361.
- Coller BS. A new murine monoclonal antibody reports an activation-dependent change in the conformation and/or microenvironment of the platelet glycoprotein IIb/IIIa. *J Clin Invest*. 1985;76:101–108.
- Coller BS, Peerschke EI, Scudder LE, Sullivan CA. Studies with a murine monoclonal antibody that abolishes ristocetin-induced binding of von Willebrand factor to platelets: additional evidence in support of GPIIb as a platelet receptor for von Willebrand factor. *Blood*. 1983;61:99–110.
- Coller BS. Platelet aggregation by ADP, collagen and ristocetin: a critical review of methodology and analysis. *Section I: Hematology*. Boca Raton, Fla: CRC Press; 1979:381–396. Schmidt RM, ed. *CRC Handbook Series in Clinical Laboratory Science*; vol I.
- Richards AH, Lubinski RM, Vanderlinde RE. Studies on the kinetic assay of lactate dehydrogenase activity. *Clin Chem*. 1975;21:1018. Abstract.
- Reisman M, Shuman B, Fei R, Dillard D, Nguyen S, Gordon L. Analysis and comparison of platelet aggregation with high-speed rotational atherectomy. *J Am Coll Cardiol*. 1997;29(suppl A):739–2. Abstract.
- Miyazaki Y, Nomura S, Miyake T, Kagawa H, Kitada C, Taniguchi H, Komiyama Y, Fujimura Y, Ikeda Y, Fukuhara S. High shear stress can initiate both platelet aggregation and shedding of procoagulant containing microparticles. *Blood*. 1996;9:3456–3464.
- Brown CH, Leverett LB, Lewis CW, Alfrey CP, Hellums JD. Morphological, biochemical and functional changes in human platelets subjected to shear stress. *J Lab Clin Med*. 1975;86:462–471.
- Kroll MH, Hellums JD, McIntire LV, Schafer AI, Moake JL. Platelets and shear stress. *Blood*. 1996;88:1525–1541.
- McCrary JK, Nolasco LH, Hellums JD, Kroll MH, Turner NA, Moake JL. Direct demonstration of radiolabeled von Willebrand factor binding to platelet glycoprotein Ib and IIb-IIIa in the presence of stress. *Ann Biomed Eng*. 1995;23:787–793.
- Mann KG, Gaffney D, Bovil EG. Molecular biology, biochemistry, and lifespan of plasma coagulation factors. In: Beutler E, Lichman MA, Coller BS, Kipps TJ, eds. *Williams Hematology*. 5th ed. New York, NY: McGraw-Hill Inc; 1995:1206–1226.
- Reverter JC, Beguin S, Kessels H, Kumar R, Hemker HC, Coller BS. Inhibition of platelet-mediated, tissue factor-induced thrombin generation by the mouse/human chimeric 7E3 antibody. *J Clin Invest*. 1996;98:863–874.
- Byzova TV, Plow EF. Networking in the hemostatic system: integrin $\alpha_{IIb}\beta_3$ binds prothrombin and influences its activation. *J Biol Chem*. 1997;272:27183–27188.
- Koch KC, Kleinhans E, Ninnemann S, Radke PW, Klues HG, Buell U, Vom Dahl J. Platelet receptor GPIIb/IIIa antagonist reduces transient ischemia during rotational atherectomy. *Circulation*. 1997;96(suppl I):I-161. Abstract.
- Dill H, Altstidl R, Regenfus M, Lehmkuhl H, Bachmann K. Doppler flow velocity measurements during coronary angioplasty. *Angiology*. 1994;45:877–882.