TIROFIBAN PROVIDES "PLATELET ANESTHESIA" DURING CARDIOPULMONARY BYPASS IN BABOONS

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Objective: Tirofiban (Aggrastat) is a reversible, nonpeptide inhibitor of platelet glycoprotein II/IIIa receptors. We tested the hypothesis that tirofiban preserves platelet number and function and shortens postoperative bleeding times in baboons after cardiopulmonary bypass. **Methods:** Four groups were studied: control, $n = 12$; low-dose tirofiban ($0.1 \mu\text{g/kg}$ per minute), $n = 7$; high-dose tirofiban ($0.3 \mu\text{g/kg}$ per minute), $n = 7$; and bolus tirofiban ($15 \mu\text{g/kg}$) followed by $0.1 \mu\text{g/kg}$ per minute during cardiopulmonary bypass, $n = 7$. After heparin, animals were perfused for 60 minutes at 50 ml/kg per minute and 37°C with a bubble oxygenator, roller pump, and peripheral cannulation. Hemodynamics, platelet count, platelet aggregation to adenosine diphosphate, and release of β -thromboglobulin were measured before tirofiban infusion, before heparin, after heparin before bypass, after 5 and 55 minutes of bypass, after protamine, and 60 minutes after protamine. Template bleeding times were measured at the same times except during cardiopulmonary bypass and 120 and 180 minutes after protamine administration. Platelet glycoprotein IIIa antigen was measured in Triton X-100 washes (Sigma Chemical Company) of the perfusion circuit after bypass. **Results:** High-dose tirofiban completely prevents platelet loss during cardiopulmonary bypass. β -Thromboglobulin release and sensitivity to adenosine diphosphate are significantly less than control at the end of bypass in all tirofiban groups. Template bleeding times return to preoperative values in both the low- and high-dose tirofiban groups 180 minutes after protamine administration and are significantly less than control bleeding times at both 120 and 180 minutes after protamine. Surface glycoprotein IIIa antigen does not significantly differ between groups. **Conclusion:** High-dose tirofiban completely preserves platelet number and improves platelet function during cardiopulmonary bypass in baboons and significantly accelerates restoration of normal template bleeding times after bypass. (J Thorac Cardiovasc Surg 1997;113:182-93)

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Bleeding remains a major problem of cardiac operations; approximately 4% of patients require reoperation within the first 24 hours to control bleeding.¹ Nonsurgical bleeding is responsible for 50% to 65% of these return visits. In addition, all patients bleed substantially more than would be expected if cardiopulmonary bypass (CPB) were not used. Bleeding problems after CPB are generally related to heparin, fibrinolysis, platelets, or a combination of these factors; deficiency of soluble coagulation factors is a rare cause of postoperative bleeding.² Inhibitors are available to manage bleeding problems related to heparin and to circulating fibrinolysins. Platelets are partially activated by heparin^{3, 4} and are diluted, destroyed, and altered during CPB because of adhesion to circuit surfaces,

Fig. 1. Heart rate, cardiac output, and mean arterial blood pressure (ABP) before, during, and after CPB for 4 groups. Circles indicate control group (group 1), triangles indicate low-dose group (group 2), squares indicate high-dose group (group 3), and diamonds indicate bolus plus low-dose group (group 4). Values are the mean $\pm$ standard error of the mean. Analysis of variance for repeated measures for four groups (statistical significance identified at $p < 0.05$, for p value or F statistic by analysis of variance). *Significant difference ($p < 0.05$, unpaired t test with Bonferroni correction) within each group as compared with BASELINE. †Significant difference ($p < 0.05$, unpaired t test with Bonferroni correction) between each tirofiban group and control.

Table I. Hemodynamic data

	Group	BASELINE	TIROFIBAN	HEPARIN	START	END
Heart rate (beats/min)	Control	109 ± 5	115 ± 25	115 ± 5	127 ± 6*	122 ± 7
	Low dose	101 ± 6	112 ± 8	118 ± 7	127 ± 8*	115 ± 7
	High dose	104 ± 3	104 ± 5	115 ± 3	133 ± 11*	103 ± 1
	Bolus + low	107 ± 7		115 ± 6	139 ± 5*	150 ± 8*†
Mean BP (mm Hg)	Control	81 ± 6	68 ± 24	75 ± 5	69 ± 5	74 ± 4
	Low dose	69 ± 5	71 ± 5	63 ± 6	55 ± 5*†	63 ± 5
	High dose	71 ± 7	70 ± 5	62 ± 4	72 ± 3	62 ± 3
	Bolus + low	59 ± 3†		74 ± 4*	64 ± 4	62 ± 4
Cardiac output (ml/min/kg)	Control	113 ± 6	114 ± 4	116 ± 7		
	Low dose	117 ± 4	116 ± 4	121 ± 4		
	High dose	112 ± 7	108 ± 10	117 ± 11		
	Bolus + low	126 ± 4		129 ± 9		

Hemodynamic measurements of heart rate, mean arterial blood pressure (BP), and cardiac output. Values are the mean ± standard error of the mean. ANOVA, Analysis of variance for repeated measures for four groups (statistical significance identified at $p < 0.05$, for p value or F statistic by ANOVA).

*Significant difference ($p < 0.05$, unpaired t test with Bonferroni correction) within each group as compared with BASELINE.

†Significant difference ($p < 0.05$, unpaired t test with Bonferroni correction) between tirofiban group and control.

aggregation and activation, and removal of damaged platelets by the reticuloendothelial system. Thus, after CPB, deficiency in platelet numbers and function prolongs template bleeding times and contributes to postoperative bleeding.

Temporary pharmacologic inhibition of platelets during the period of CPB is an attractive strategy for preserving platelet number and function. Phosphodiesterase inhibitors (dipyridamole) and cyclic adenosine monophosphate catalysts (prostanoids) reversibly inhibit platelets, and both have been used clinically to preserve platelet number and function.⁵ However, dipyridamole given intravenously only partially protects platelets during CPB⁵ and has a long half-life in plasma. The drug has not progressed to general use. Prostanoids, particularly iloprost (Berlex Laboratories, Inc.), protect platelets during CPB, but must be used with a vasoconstrictor to maintain systemic blood pressure.⁶ Aspirin inhibits cyclooxygenase and causes irreversible inhibition of platelet thromboxane and prostacyclin synthesis.

The final step in platelet adhesion and aggregation is binding fibrinogen to the activated, membrane-bound glycoprotein complex, glycoprotein (GP) IIb/IIIa.^{7,8} Antibodies directed against GPIIb/IIIa and the disintegrin family of RGD (arginyl-glycyl-aspartyl)-containing peptides⁹⁻¹¹ are potent inhibitors of these reactions. Previous studies from our laboratories demonstrated that four disintegrins—bitistatin, echistatin, albolabrin, and flavoridin—protect platelets during simulated extracorporeal circulation of fresh heparinized human blood by preventing platelet adhesion and aggregation.¹² A single dose of bitistatin protected platelets during 12

to 14 hours of extracorporeal membrane oxygenation in sheep.¹³

This study evaluates a novel, reversible, nonprotein platelet GPIIb/IIIa receptor inhibitor, tirofiban (Aggrastat, Merck Inc.). Tirofiban is a tyrosine derivative and has been designed as a small molecular, nonpeptide RGD mimetic recognized by the GPIIb/IIIa receptor complex but not by other RGD integrins.^{14,15} The drug is nontoxic and has been tested in the dog and baboon, in healthy human volunteers,¹⁶ and in patients with coronary artery disease. The drug has a half-life in plasma of 1.6 hours.¹⁷ These studies reveal that tirofiban is effective in inhibiting platelet aggregation, in increasing bleeding times, and in preventing arterial thrombus formation in a dose-dependent manner without significant drug-related adverse effects.

We tested the efficacy of tirofiban to preserve both platelet numbers and function during CPB and to shorten postoperative bleeding times so that adequate numbers of healthy platelets are available after CPB for hemostasis.

Methods

Juvenile baboons (*Papio anubis*) weighing 10 to 23 kg were used. The animals were divided into four groups: control (group 1, $n = 12$); low-dose tirofiban infusion (0.1 $\mu\text{g/kg}$ per minute) during the hour before CPB and during 60 minutes of CPB (group 2, $n = 7$); high-dose tirofiban infusion (0.3 $\mu\text{g/kg}$ per minute) before and during CPB, as in group 2 (group 3, $n = 7$); and bolus injection of tirofiban (15 $\mu\text{g/kg}$) before starting CPB and low-dose infusion (0.1 $\mu\text{g/kg}$ per minute) during CPB (group 4, $n = 7$). For each CPB experiment, the baboon was placed in a squeeze cage and sedated with ketamine hydrochloride, 10 mg/kg, given intramuscularly. Anesthesia was induced

PROTAMINE	PROTAMINE-60	p Value (ANOVA)	F-statistic (ANOVA)
99 ± 3	97 ± 4		0.0001
98 ± 4	96 ± 3	0.53671	
97 ± 1	97 ± 1	0.32198	
124 ± 8†	109 ± 5†	0.000001	
66 ± 3*	63 ± 3*		0.002
63 ± 3	60 ± 2	0.0005	
60 ± 3	59 ± 3	0.00967	
65 ± 4	61 ± 2	0.00837	
114 ± 7	100 ± 5		0.0001
136 ± 4*†	107 ± 4	0.0401	
116 ± 11	102 ± 7	0.83242	
144 ± 5†	125 ± 6†	0.00004	

with thiopental sodium, 5 mg/kg, given intravenously. The animal was intubated, and general anesthesia was maintained with inhalational isoflurane. The right or left side of the neck and both groins were prepared and draped appropriately for sterile cutdown and cannulation of vessels. Hemodynamic monitoring was accomplished by means of an arterial line with a 22-gauge catheter placed in the femoral artery and a 5F thermodilution catheter placed via a femoral vein. After anticoagulation with porcine sodium heparin (300 units/kg, Elkins-Sinn, Inc., Cherry Hill, N.J.) a 10F to 14F Bio-Medicus (Medtronic Bio-Medicus, Eden Prairie, Minn.) wire-wrapped, polyurethane catheter was introduced into the jugular vein and advanced into the right atrium. A similar, but shorter 8F arterial catheter for reinfusion was inserted into the femoral artery.

Each bypass circuit was assembled with silicone rubber tubing (Dow Corning Corporation, Midland, Mich.) incorporating a bubble oxygenator (Bentley 5/Pediatric, Baxter Healthcare Corporation, Irvine, Calif.), an arterial filter (Intersept Pediatric, Medtronic, Inc., Anaheim, Calif.), and a roller pump (Sarns model 13400, Sarns/3M, Ann Arbor, Mich.). The circuit was primed with approximately 500 ml of Normosol solution (Abbott Laboratories, North Chicago, Ill.). Normothermic CPB began at a flow rate of 50 ml/kg per minute (approximately one half of the baboon's resting cardiac output), and perfusion was maintained for 60 minutes. In groups 2, 3, and 4, tirofiban was given at a constant flow rate by means of an infusion pump (model 55-1111, Harvard Apparatus Co., South Natick, Mass.) through the femoral vein.

Seven blood samples (15 to 20 ml each) were obtained at baseline before tirofiban infusion (time point labeled baseline); after the start of tirofiban infusion in groups 1, 2, and 3 before heparin (tirofiban); after heparin in groups 1, 2, and 3 or after heparin and tirofiban injection in group 4 before CPB (heparin); 5 minutes after the start of CPB (start); 5 minutes before CPB was stopped (end); 10 minutes after protamine was given (3 mg/kg, Elkins-Sinn, Inc., Cherry Hill, N.J.) (protamine); and 60 minutes after time point protamine (protamine-60).

Heart rate by electrocardiogram, systemic (systolic, diastolic, mean) arterial blood pressures, central venous pressure, pulmonary arterial pressure, and pulmonary

capillary wedge pressure were continuously monitored. Intermittent thermodilution cardiac outputs were measured before and after CPB. Blood samples were assayed for hematocrit value, platelet count, white blood cell count, platelet aggregation to adenosine diphosphate, and β -thromboglobulin (β TG) release. Dilution of formed blood elements and plasma β TG was corrected using the hematocrit value. The total amount of blood withdrawn was limited to less than 10% of body weight (120 to 180 ml) for each experiment, and blood remaining in the perfusion circuit was reinfused at the end of CPB. The CPB circuit was then rinsed with 500 ml normal saline solution followed by 500 ml of 0.1% Triton X-100 solution (Sigma Chemical Company, St. Louis, Mo.). An aliquot of the Triton X-100 wash was taken for measurement of surface platelet GPIIIa antigen. Plasma and Triton X-100 wash samples were frozen at -70°C until analysis. At least 6 weeks of recovery for each baboon was allowed before the next experiment.

Hematocrit and platelet count assays were performed on whole blood. Platelets were counted by phase microscopy or by means of a Coulter Counter (model STKR, Coulter Electronics Inc., Hialeah, Fla.) in triplicate. Platelet count was corrected for hemodilution and was expressed as a percentage of BASELINE values. Platelet aggregation to adenosine diphosphate was studied with a Payton aggregometer (model 440, Chrono-Log, Inc., Havertown, Pa.). Platelet-rich plasma and platelet-poor plasma were prepared from citrated blood (10 ml with 10% by volume of 3.8% citrate) by differential centrifugation at 150g for 10 minutes and 13,600g for 5 minutes, respectively. Before studies of aggregation, the platelet count of platelet-rich plasma was adjusted to 150,000/ μl by dilution with platelet-poor plasma. The concentration of adenosine diphosphate required to produce complete second-wave aggregation was measured; complete second-wave aggregation was assumed when light transmission was 62.5% or greater within 5 minutes.¹⁸ Platelet aggregation is reported as a percentage, normalized to the concentration of adenosine diphosphate required to obtain full aggregation of the baseline sample and the percent aggregation observed at that adenosine diphosphate concentration in subsequent samples.

Template bleeding times were measured in duplicate on the forearm at the same time points as blood samples except during CPB and also were measured 120 and 180 minutes after PROTAMINE (time points labeled PROTAMINE-120 and PROTAMINE-180) by means of a blood pressure cuff inflated to 40 mm Hg. The Simplate II (Organon Teknica Corporation, Durham, N.C.) lancet was used to create reproducible skin incisions for determinations of bleeding times.

For plasma β TG analysis blood was withdrawn into centrifugation tubes containing 10% (by volume) of 3.8% acid-citrate-dextrose and prostaglandin E_1 solution at 0°C . β TG was measured by radioimmunoassay.¹⁹ Data for β TG were corrected for hemodilution.

For measurement of platelet GPIIIa antigen eluted in Triton X-100 wash, enzyme-linked immunosorbent assay plates were coated with a 400 nmol/L solution of ristostatin (200 μmol /well) in 0.05 mol/L carbonate/bicarbonate buffer (pH, 9.2) and incubated at 4°C overnight. Eris-

Table II. Measured blood and plasma constituents during experiments

	Group	BASELINE	TIROFIBAN	HEPARIN	START	END
Platelet count (% of baseline)	Control	100	95.4 ± 0.5	87.4 ± 3.7*	91.8 ± 3.0	75.9 ± 5.2*
	Low dose	100	94.4 ± 2.2	85.4 ± 2.6*	82.0 ± 3.6*†	78.9 ± 6.2*
	High dose	100	99.2 ± 2.1	92.3 ± 3.8	104.3 ± 4.0†	115.9 ± 6.0*†
	Bolus + low	100		98.5 ± 2.3†	91.7 ± 3.4	91.3 ± 6.1
Aggregation to ADP (% of baseline)	Control	100	117.9 ± 0.0	105.6 ± 6.2	74.0 ± 9.7*	81.3 ± 6.4*
	Low dose	100	110.3 ± 7.5	89.9 ± 7.3	54.1 ± 4.2*	56.2 ± 1.7*†
	High dose	100	37.9 ± 4.8*†	34.2 ± 3.5*†	12.8 ± 2.3*†	12.7 ± 2.5*†
	Bolus + low	100		51.7 ± 7.7*†	56.5 ± 4.8*	60.4 ± 6.2*†
βTG (ng/ml, Hct. corrected)	Control	15.1 ± 7.8	19.3 ± 0.8	24.8 ± 5.3	80.2 ± 15.3*	166.7 ± 11.1*
	Low dose	31.3 ± 2.7	26.8 ± 5.0	27.5 ± 5.7	55.8 ± 7.5	125.9 ± 7.8*†
	High dose	24.5 ± 4.7	36.2 ± 3.5	36.8 ± 3.1	34.2 ± 6.9†	70.6 ± 9.2*†
	Bolus + low	19.2 ± 4.9		19.5 ± 2.4	40.7 ± 11.8†	127.3 ± 10.8*†
Bleeding times (min)	Control	3.9 ± 0.1	4.6 ± 0.1	5.5 ± 0.5		
	Low dose	3.7 ± 0.2	5.6 ± 0.5	11.0 ± 1.8*†		
	High dose	4.1 ± 0.2	10.9 ± 1.6*†	19.5 ± 0.3*†		
	Bolus + low	3.6 ± 0.2		17.8 ± 0.8*†		
Hematocrit (%)	Control	34.8 ± 0.8	32.5 ± 3.5	34.1 ± 0.8	15.6 ± 0.5*	20.8 ± 0.8*
	Low dose	32.4 ± 0.8†	33.0 ± 0.8	32.3 ± 0.7	15.7 ± 0.5*	20.6 ± 0.8*
	High dose	35.1 ± 0.7	34.4 ± 0.8	33.0 ± 0.5	16.0 ± 0.8*	20.7 ± 0.6*
	Bolus + low	32.4 ± 0.6†		31.9 ± 0.3†	17.7 ± 0.7*†	20.2 ± 0.7*
WBC count (% of baseline)	Control	100	104.3 ± 2.6	116.2 ± 4.0	102.8 ± 13.3	222.5 ± 29.7*
	Low dose	100	106.8 ± 7.7	132.0 ± 10.1	75.2 ± 7.2	248.8 ± 26.7*
	High dose	100	103.8 ± 5.7	118.4 ± 8.0	94.0 ± 13.1	260.0 ± 38.9*
	Bolus + low	100		109.5 ± 5.9	96.1 ± 12.8	223.4 ± 16.4*

Changes in measured blood and plasma constituents and bleeding times during the each experiment. Values are the mean ± standard error of the mean. ADP, Adenosine diphosphate; βTG, beta-thromboglobulin; WBC, white blood cell; ANOVA, analysis of variance for repeated measures for four groups (statistical significance identified at $p < 0.05$, for p value or F statistic by ANOVA).

*Significant difference ($p < 0.05$, unpaired t test with Bonferroni correction) within each group as compared with BASELINE.

†Significant difference ($p < 0.05$, unpaired t test with Bonferroni correction) between each tirofiban group and control.

tostatin was prepared as described earlier.²⁰ To block nonreacted surfaces, plates were incubated for 60 minutes at 37° C with 250 μl/well phosphate-buffered saline solution (PBS)/0.5% Tween 20 containing 5% nonfat milk. After being washed (three times) with PBS/Tween buffer, samples were added into wells in 1:2 dilution in buffer containing 20 nmol/L Tris-HCL (pH 7.4), 150 mmol/L NaCl, 2 nmol/L CaCl₂, 2 mmol/L MgCl₂, and 1% bovine serum albumin and were incubated for 60 minutes at 37° C. Subsequently, known concentrations of purified GPIIb/IIIa were added to the wells on the same plate to obtain the standard curve. The plate was washed three times with PBS/Tween buffer, and aliquots of 400 ng monoclonal antibody AP3 (recognizing GPIIIa) in PBS/Tween buffer containing 3% nonfat milk were added to each well. Plates were incubated for 60 minutes at 37° C and then washed three times with PBS/Tween buffer. After washing, the binding of AP3 was detected with the use of alkaline phosphatase-conjugated goat antimouse immunoglobulin G as described.²¹

Data points represent the mean ± standard error of the mean of measurements. The unpaired Student's t test with Bonferroni correction and analysis of variance for repeated measures (SPSS for Windows 6.1, Chicago, Ill.) were used for statistical analysis of differences between groups, and the paired Student's t test with Bonferroni correction was used for analysis of differences within the groups. Differences

were considered statistically significant at the $p < 0.05$ level. This study was approved by the University of Pennsylvania Committee on Animal Care and Utilization.

Results

Table I shows hemodynamic measurements of heart rate, mean arterial blood pressure, and cardiac output. By analysis of variance, as compared with control at various times, mean arterial pressure was significantly higher in animals in groups 2, 3, and 4 and heart rate was also significantly higher in group 4 baboons (Fig. 1). Cardiac outputs were significantly higher in both low-dose tirofiban groups (groups 2 and 4) than in the control group. Although minor differences within and occasionally between groups were observed, hemodynamic measurements remained within the normal range for all doses of tirofiban at all sampling times.

Changes in measured blood and plasma constituents and bleeding times during the experiments are listed in Table II. In all studies dilution of the pump priming volume reduced hematocrit value in all groups similarly during perfusion. Hematocrit value

PROTAMINE	PROTAMINE-60	PROTAMINE-120	PROTAMINE-180	p Value (ANOVA)	F statistic (ANOVA)
57.9 ± 2.9*	62.6 ± 2.9*				0.0001
61.5 ± 4.4*	67.4 ± 4.6*			0.97339	
81.0 ± 8.0*†	89.1 ± 6.9†			0.000001	
72.3 ± 7.3*	79.1 ± 5.3*†			0.00009	
72.5 ± 5.5*	74.8 ± 3.4*				0.0001
63.0 ± 6.4*	75.3 ± 7.2*			0.00027	
45.2 ± 6.9*†	72.5 ± 4.9*			0.000001	
58.7 ± 6.9*	82.1 ± 4.7*			0.000001	
49.2 ± 5.6*	15.7 ± 5.1				0.009
60.5 ± 9.9*	22.5 ± 10.9			0.41537	
59.9 ± 15.7*	32.5 ± 8.5			0.00379	
43.7 ± 9.6*	15.9 ± 2.2			0.00776	
13.6 ± 1.5*	11.3 ± 1.3*	10.6 ± 1.7*	8.7 ± 1.7*		0.0001
17.0 ± 0.8*	12.0 ± 1.5*	6.8 ± 0.5*†	4.9 ± 0.3†	0.62563	
17.3 ± 1.1*	13.5 ± 1.3*	7.4 ± 0.6*†	5.4 ± 0.4†	0.00046	
16.2 ± 1.6*	13.7 ± 1.9*	7.9 ± 0.9*	6.5 ± 0.6	0.00172	
27.4 ± 0.6*	27.7 ± 0.6*				0.009
26.0 ± 0.6*	26.9 ± 0.6*			0.00693	
26.6 ± 1.0*	27.1 ± 1.1*			0.43865	
25.1 ± 0.3*†	26.4 ± 0.5*			0.00552	
171.0 ± 20.9*	247.7 ± 26.9*				0.228
182.5 ± 21.6*	263.2 ± 30.0*			0.56180	
154.5 ± 30.4	240.4 ± 38.9*			0.92351	
239.7 ± 23.7*†	332.5 ± 32.4*			0.04976	

rose after CPB ended when blood was reinfused from the perfusion circuit (Fig. 2).

Platelet counts decreased significantly during and after CPB in the control and both low-dose groups of animals (groups 1, 2, and 4). The bolus injection and low-dose infusion of tirofiban better preserved platelet counts than the low-dose infusion alone, but neither dosage scheme completely prevented platelet adhesion and loss as well as high-dose tirofiban. High-dose tirofiban completely prevented platelet adhesion during CPB and also most strongly inhibited platelet function as assessed by sensitivity to adenosine diphosphate. As shown in Table II, platelet counts and sensitivity to adenosine diphosphate significantly differed from control baboons during and after CPB in animals that received high-dose tirofiban. However, platelet function in group 3 did not return to control values until 60 minutes after protamine (Fig. 3).

Tirofiban partially inhibited β TG release, and the degree of inhibition was dose-related (Fig. 4). High-dose and bolus plus low-dose tirofiban significantly reduced plasma β TG during CPB. Animals receiving low-dose tirofiban (group 2) also had significantly less plasma β TG at the end of CPB than control baboons. Plasma β TG was rapidly cleared

and concentrations returned to baseline within the hour after protamine administration.

As expected, tirofiban prolonged bleeding times and this effect was dose-related (Fig. 5). The addition of heparin further prolonged bleeding times before the start of CPB. After CPB all doses of tirofiban hastened the return of bleeding times toward normal, and bleeding times in both low-dose and high-dose animals (groups 2 and 3) were significantly less than control 2 and 3 hours after protamine was given.

White blood cell count increased after CPB in all groups and there were no consistent differences between groups. Surface GPIIIa antigen washed from the perfusion circuit after CPB was 2.75 ± 0.42 ng/100 μ l in the control group and did not significantly differ between groups 2 and 3 (low dose, 4.04 ± 0.31 ng/100 μ l; high dose, 3.14 ± 0.40 ng/100 μ l). Bolus plus low dose (group 4) was significantly ($p < 0.05$) less than control (0.44 ± 0.31 ng/100 μ l).

Discussion

The concept of "platelet anesthesia," introduced with the use of prostaglandin E_1 in rhesus monkeys,²² postulates that complete, but reversible, in-

Fig. 2. Hematocrit-corrected platelet counts before, during, and after CPB for four groups. Platelet counts are standardized as a percentage of BASELINE for each time point of the group. *Circles* indicate control group (group 1), *triangles* indicate low-dose group (group 2), *squares* indicate high-dose group (group 3), and *diamonds* indicate bolus plus low-dose group (group 4). Values are the mean $\pm$ standard error of the mean. Analysis of variance for repeated measures for four groups (statistical significance identified at $p < 0.05$, for p value or F statistic by analysis of variance). *Significant difference ($p < 0.05$, unpaired t test with Bonferroni correction) *within* each group as compared with BASELINE. †Significant difference ($p < 0.05$, unpaired t test with Bonferroni correction) *between* each tirofiban group and control.

hibition of platelet reactivity during CPB can prevent platelet aggregation, release, and adhesion and thus preserve platelet number and function for hemostasis after CPB ends. During CPB, platelets are activated by heparin^{3,4} and by contact with the nonendothelial cell, biomaterial surfaces of the perfusion circuit.^{3,12} Inhibition of platelets during this portion of the operation does not adversely affect the operation because blood is anticoagulated and is conserved by means of mechanical measures. The key requirements for a "platelet anesthetic" are

complete inhibition of all platelet functions just before heparin is given and quick and complete reversal of this inhibition after CPB ends and protamine is given.

There are many platelet inhibitors. Aspirin only partially inhibits platelet reactivity and is not reversible. Dipyridamole, a reversible, phosphodiesterase inhibitor, only partially inhibits platelets during CPB.⁵ The prostanoids, particularly the prostacyclin analog, iloprost (Berlex Labs), incompletely inhibit platelet membrane receptors and are reversible, but

Fig. 3. Platelet aggregation to adenosine diphosphate (ADP) before, during, and after CPB for four groups. Data points are standardized as a percentage of BASELINE for each time point of the group. Circles indicate control group (group 1), triangles indicate low-dose group (group 2), squares indicate high-dose group (group 3), and diamonds indicate bolus plus low-dose group (group 4). Values are the mean $\pm$ standard error of the mean. Analysis of variance for repeated measures for four groups (statistical significance identified at $p < 0.05$, for p value or F statistic by analysis of variance). *Significant difference ($p < 0.05$, unpaired t test with Bonferroni correction) within each group as compared with BASELINE. †Significant difference ($p < 0.05$, unpaired t test with Bonferroni correction) between each tirofiban group and control.

also are such powerful vasodilators that their use is impractical.⁶

Disintegrins are RGD peptides that prevent fibrinogen binding to platelets by reversibly inhibiting the platelet GPIIb/IIIa receptor complex.¹⁰ Natural RGD peptides are potent, reversible inhibitors of platelet aggregation and cellular adhesion that are effective in extracorporeal perfusion systems.^{12, 13} The chimeric compound c7E3 containing a murine antibody fragment irreversibly inhibits the GPIIb/

IIIa receptor and is approved for clinical use. RGD peptidomimetics and cyclic RGD are synthetic GPIIb/IIIa antagonists²³; several of these compounds, including tirofiban, are potent, reversible, nontoxic inhibitors of platelet aggregation. These drugs effectively inhibit platelet reactivity during interventional cardiology, and results of clinical trials show few side effects and no toxicity.^{16, 23} However, in these applications prompt reversibility of the drug is not required and indeed is often

Fig. 4. Hematocrit corrected β TG release before, during, and after CPB for four groups. Circles indicate control group (group 1), triangles indicate low-dose group (group 2), squares indicate high-dose group (group 3), and diamonds indicate bolus plus low-dose group (group 4). Values are the mean $\pm$ standard error of the mean. Analysis of variance for repeated measures for four groups (statistical significance identified at $p < 0.05$, for p value or F statistic by analysis of variance). *Significant difference ($p < 0.05$, unpaired t test with Bonferroni correction) within each group as compared with BASELINE. †Significant difference ($p < 0.05$, unpaired t test with Bonferroni correction) between each tirofiban group and control.

undesirable until remodeling and healing of the vascular injury site begins. For cardiac operations, prompt and complete reversal of platelet inhibition is an absolute requirement.

The results of these studies show that high-dose tirofiban prevents platelet adhesion and aggregation, attenuates α granule release, and accelerates the return of normal postoperative bleeding times. The drug has no discernible side effects on hemodynamics, is in phase III clinical trials, and has no known toxicity. This drug appears to be a promising

“platelet anesthetic” for clinical trials during cardiac operations.

However, tirofiban may not be the ideal drug for cardiac operations. The half-life in plasma (1.6 hours)¹⁷ and lack of a mechanism to immediately reverse its action mean bleeding times are still prolonged during the critical first few hours after operation when most postoperative bleeding occurs. Furthermore, the drug does not completely inhibit β TG release or surface-adsorbed GPIIIa antigen. Because the drug does not inhibit the platelet

Fig. 5. Template bleeding times before, during, and after CPB for four groups. *Circles* indicate control group (group 1), *triangles* indicate low-dose group (group 2), *squares* indicate high-dose group (group 3), and *diamonds* indicate bolus plus low-dose group (group 4). Values are the mean $\pm$ standard error of the mean. Analysis of variance for repeated measures for four groups (statistical significance identified at $p < 0.05$, for p value or F statistic by analysis of variance). *Significant difference ($p < 0.05$, unpaired t test with Bonferroni correction) *within* each group as compared with BASELINE. †Significant difference ($p < 0.05$, unpaired t test with Bonferroni correction) *between* each tirofiban group and control.

thrombin receptor and because thrombin is formed and circulates during extracorporeal perfusion,²⁴ platelets probably are activated even though aggregation and adhesion are inhibited.

A combination of tirofiban with a low dose of iloprost, insufficient to cause vasodilatory side effects,²⁵ may overcome this limitation. On the other hand, a shorter-acting, more rapidly metabolized drug may sufficiently protect platelets to produce normal coagulation without a synergistic inhibitor. Some cyclic RGD peptides have shorter half-life

times; after an infusion of integrilin is stopped, bleeding times return to normal within 15 minutes.²⁶ Reilly and associates²⁷ have developed a monoclonal antibody against the cyclic RGD peptide DMP 728 that immediately neutralizes its effect on bleeding time in dogs. The goal is to produce a normal bleeding time at the time protamine is given. Nevertheless, tirofiban should produce a meaningful decrease in postoperative bleeding after cardiac operations, particularly in patients who require reoperation or prolonged procedures.

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Discussion

Dr. Ben P. Bidstrup (Townsville, Australia). Dr. Edmunds' group is to be congratulated for their continuing efforts in elucidating the mechanisms of coagulation disturbance during CPB, and for their efforts to examine other agents that may reduce these changes. It is important that we make a distinction between platelet inhibition (as achieved by a drug such as tirofiban) and platelet activation. Activation means release of granule substances and with subsequent irreversible changes such that these platelets no longer can play a role in hemostasis. Tirofiban may be an important agent that will allow platelet inhibition in the clinical setting.

How did you arrive at the dosage regimen? You have described several doses. I am reminded of the troubles

that clinicians have had trying to achieve an appropriate dose of aprotinin, another agent that is effective in reducing postoperative bleeding. Given the half-life of 1½ hours of tirofiban, how do you think platelet anesthesia will be reversed in human beings? Surgeons, as a general rule, are unlikely to want to wait 2 hours for the bleeding time and probably bleeding, to return to normal. How did you measure the surface GPIIb/IIIa antigen?

Dr. Hiramatsu. Thank you for the question. We measured surface GPIIIa antigen in the Triton X-100 wash sample from rinsing the extracorporeal perfusion circuit. Using an enzyme-linked immunosorbent assay for surface-adsorbed GPIIIa antigen, we did not see any differences in adsorbed GPIIIa between control and high- and low-dose tirofiban.

Dr. Bidstrup. That is not actually going to show whether there are any changes on the surface of the platelet, and it may be better to use flow cytometry to look at that. You are really looking at whether the IIb/IIIa has been cleaved from the surface of the platelet with that method, aren't you?

Dr. Edmunds. The GPIIIa antigen is an assay developed by Dr. Niewiarowski, which measures the platelet receptor that is adsorbed onto the tubing. At the end of CPB the circuit is rinsed with saline solution and washed with Triton X-100 solution. The GPIIIa antigen is measured in the Triton X-100 wash.

The results are very interesting and became available about a week ago. There is much less surface-adsorbed

GPIIIa antigen in the bolus low-dose group than in the other three groups—control, high dose, and low dose. We cannot explain this result at this point.

Regarding the bleeding time question, tirofiban quickens the restoration of a normal bleeding time. The duration of a prolonged bleeding time is still longer than we wish, so tirofiban is not the ideal agent. We are looking at an agent with a half-life of about 15 minutes.

This receptor inhibitor does not completely prevent platelet activation. There may be a need to add a cyclic adenosine monophosphate elevator in addition to a GPIIb/IIIa receptor inhibitor. Nevertheless, we believe we are close to developing a platelet anesthetic for CPB.

Dr. James K. Kirklin (*Birmingham, Ala.*). Do you have any information about its safety or effectiveness with repeated exposures? Is there any tendency for tachyphylaxis?

Dr. Hiramatsu. Several animals were studied repeatedly without any noticeable change in results or hemodynamics. Tirofiban is not a protein and therefore is less likely to cause anaphylaxis.

Dr. Edward D. Verrier (*Seattle, Wash.*). If there is an underlying ischemic-reperfusion event, is there any danger that this drug will exacerbate the reperfusion injury with this number of platelets?

Dr. Hiramatsu. We don't have an answer for your question and did not investigate the possibility in these studies.