Pharmacologic platelet anesthesia by glycoprotein IIb/IIIa complex antagonist and argatroban during in vitro extracorporeal circulation

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Objective: Contact between blood and the synthetic surfaces of a cardiopulmonary bypass circuit leads to platelet activation, and resultant platelet dysfunction contributes to postoperative bleeding. We compared the effects of various platelet inhibitors on preservation of platelet function during simulated cardiopulmonary bypass circulation.

Methods: Fresh human blood was recirculated in an in vitro cardiopulmonary bypass model circuit. We measured various platelet activation markers including expressions of PAC-1 and P-selectin, annexin V binding, and microparticle formations by means of whole-blood flow cytometry.

Results: Two types of glycoprotein IIb/IIIa complex antagonists, peptide-mimetic FK633 and abciximab and prostaglandin E₁, significantly prevented platelet loss and the increase in binding of PAC-1, an antibody specific for fibrinogen receptor on activated platelets, during extracorporeal circulation of heparinized blood. These antagonists significantly suppressed but did not abolish P-selectin expression, annexin V binding, and microparticle formation. Anti-von Willebrand factor monoclonal antibody and aurin tricarboxylic acid (an inhibitor of glycoprotein Ib) had no effect on platelet activation during simulated cardiopulmonary bypass circulation. These data suggest that inhibition of fibrinogen binding glycoprotein IIb/IIIa complex is partly effective in attenuating platelet activation in a heparinized cardiopulmonary bypass model circuit. The direct thrombin inhibitor argatroban prevented platelet loss and expression of P-selectin significantly more than did heparin. A combination of FK633 with argatroban as a substitute for heparin further prevented platelet loss and platelet secretion during simulated cardiopulmonary bypass circulation, although the inhibition of microparticle formation was less.

Conclusion: The inhibition of both platelet adhesion and thrombin may be effective to preserve platelet number and function during cardiopulmonary bypass circulation.

Platelet consumption and dysfunction during cardiopulmonary bypass (CPB) result from contact activation with synthetic surfaces of CPB circuits and the shear stress of the flowing blood.¹⁻³ The bleeding tendency during and after cardiac operations with CPB is largely caused by platelet dysfunction.⁴ Platelet dysfunction has been reproduced in vitro in recirculating heparinized human blood in a CPB model circuit composed of a tube and reservoir with a roller-head pump.^{1,5} We have

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shown that the heparin-coated circuit maintains platelet aggregability in the in vitro extracorporeal circulation.⁵ Temporary pharmacologic inhibition of platelets (platelet anesthesia⁶) during CPB is another potential strategy for preserving platelet number and function. We tested the effects on platelet consumption and functions during in vitro heparinized CPB circulation of various platelet inhibitors, including FK633 (a peptide-mimetic glycoprotein [Gp] IIb/IIIa complex antagonist),⁷ abciximab (Fab fragment of antibody against GpIIb/IIIa complex),^{8,9} an anti-von Willebrand factor (vWF) monoclonal antibody (AJvW-2),¹⁰ aurotricarboxylic acid (ATA, an inhibitor of platelet GpIb),^{11,12} and prostaglandin E₁ (PGE₁). To assess platelet activation during blood-synthetic surface contact, we quantitated platelet functional and structural alterations during a simulated CPB circulation with heparin used as an anticoagulant by means of whole-blood flow cytometry.

Platelets can be activated not only by contact activation with an in vitro CPB model circuit but also by coming in contact with thrombin, produced as a terminal event in coagulation cascades.⁵ Anticoagulation with heparin during CPB circulation does not entirely prevent surface-induced coagulation and the adherence of blood cells to membranes, even though heparin is the anticoagulant generally used in extracorporeal circulations (hemodialysis and CPB). The use of heparin can lead to other complications, including platelet dysfunction, thrombocytopenia, and lipid abnormalities, all predisposing toward cardiovascular disorders.¹³ Argatroban, a small-molecule synthetic direct thrombin inhibitor, binds rapidly and reversibly to both clot-bound and soluble thrombin.¹⁴ Because the utility of combining a direct thrombin inhibitor and a GpIIb/IIIa complex inhibitor has not been fully explored, we wanted to determine the efficacy of combining argatroban and FK633 to enhance inhibition of platelet activation during simulated extracorporeal circulation.

Material and Methods

Reagents

FK633, N-[4-(4-amidinophenoxy)butyryl]-a-L-aspartyl-L-valine, was provided by Fujisawa Pharmaceutical (Fujisawa Pharmaceutical Co, Ltd, Osaka, Japan), abciximab (Reopro) was purchased from Eli Lilly (Eli Lilly and Company, Indianapolis, Ind), and PGE₁ was provided by Ono Pharmaceutical (Ono Pharmaceutical Co, Osaka, Japan). AJvW-2 was provided by Ajinomoto Pharmaceutical (Ajinomoto Pharmaceutical Co, Tokyo, Japan). ATA was purchased from Sigma (Sigma, St Louis, Mo), and low-molecular weight ATA polymers (molecular weight <5 kd) were collected at 4°C with a 5-kd cutoff filter (Ultrafree-4; Millipore Corporation, Bedford, Mass). Argatroban was provided by Mitsubishi Pharma (Mitsubishi Pharma Co, Osaka, Japan).

Preparation of the Model of CPB

The model circuits (surface area 0.01 m²) were assembled from standard medical-grade polyvinylchloride tubing (diameter 3.0

mm) with polycarbonate connectors and reservoirs.⁵ These model circuits did not connect with oxygenators or air filters. Blood was drawn by venipuncture without stasis from informed volunteer staff members with a 17-gauge needle or taken into an ethylenediaminetetraacetic acid-treated tube for full blood count. Blood samples were diluted to approximate bypass hematocrit (25%) with priming solution (Veen D; Nikkenkagaku, Tokyo, Japan). Heparin chloride was injected in a standard heparin dose used in clinical CPB (3 U/mL). In each experimental study (n = 5), FK633 (100 and 500 nmol/L), abciximab (2 and 10 µg/mL), ATA (200 mg/µL), or AJvW-2 (3.0 µg/mL) was added to the reservoir just before circulation. In some experiments with 500-nmol/L FK633 (n = 5), argatroban (3 µg/mL) was added to the circuit reservoir as a substitute for heparin. The blood priming solution was recirculated by a precisely shimmed, barely occlusive calibrated roller pump (Senko Medical Co, Ltd, Osaka, Japan) for 3 hours at a rate of equal to twice the circulating volume per minute (100 mL/min). Blood temperature was maintained at approximately 28°C. Shear stress (γ) was calculated as follows: $\gamma = 4\eta Q/\pi r^3$, where η is viscosity, Q is blood flow, and r is tube radius. Shear stress in the model circuit was approximately 30 dyne/cm².

Blood Sampling and Platelet Preparation

Samples were taken at six times: (1) before circulation; (2) 5 minutes, (3) 60 minutes, (4) 120 minutes, and (5) 180 minutes after beginning circulation; and (6) 120 minutes after the completion of circulation. Whole blood samples were separated into two parts; one mixed with ethylenediaminetetraacetic acid was analyzed for platelet counting with Sysmex KX-21 (Sysmex Co, Kobe, Japan), whereas the other was immediately mixed with 1 volume of 3.8% sodium citrate solution to 9 volumes of whole blood for flow cytometry.

Flow Cytometry of Activated Platelets and Platelet-Derived Microparticles

We detected activated platelets in whole blood with a modification of the whole-blood flow cytometry method,¹⁵⁻¹⁷ as described in detail here. The following monoclonal antibodies were used for this study: SZ2 (Immnotech, Marseilles, France), which reacts with the vWF-binding site of the α -chain of GpIb; SZ22 (Immnotech), which reacts with GpIIb; CLB-Thromb/6 (Immnotech), which reacts with P-selectin of α -granule membrane; and PAC-1 (BD Biosciences, San Jose, Calif), which reacts with activated fibrinogen receptor GpIIb/IIIa complex.¹⁵ Increased expression of P-selectin is indicative of α -granule secretion. Whole blood containing platelets (about 1.0×10^6) was incubated with 10 µL fluorescein isothiocyanate-conjugated (FITC) antibody for 30 minutes at room temperature and in the dark. Samples were fixed in 1% paraformaldehyde (pH 7.3) at 4°C and analyzed with an Ortho Cytron Absolute flow cytometer (Ortho Diagnostic Systems, Tokyo, Japan). For each sample a gate was drawn around the platelet population, and the fluorescence signal from 10,000 individual cells was measured with the gain setting in logarithmic mode. When measuring GpIIb and GpIb, which were strongly expressed on all platelets at all times, the data were expressed as mean fluorescence intensity. The "flip-flop" of the platelet membrane, leading to the exposure of negatively charged phospholipids (predominantly phosphatidylserine) in platelet activation, was

Figure 1. Effects of FK633, abciximab, and PGE₁ on platelet counts; expressions of platelet surface GpIb, PAC-1, P-selectin, and annexin V; and PMP formation during simulated CPB circulation. Heparinized (3 U/mL) blood was recirculated with vehicle (control, filled circles) or with 500-nmol/L FK633 (filled triangles), 2- μ g/mL abciximab (open squares), or 50-ng/mL PGE₁ (open circles). Data points represent mean; error bars represent SD (n = 5). Plus signs indicate $P < .05$, asterisks indicate $P < .01$, and double asterisks indicate $P < .001$.

studied with FITC annexin V.^{18,19} Annexin V binding is indicative of phosphatidylserine exposure, which results in platelets with an increased procoagulant tendency. Platelets were incubated with FITC annexin V (BD PharMingen, San Diego, Calif) for 15 minutes at room temperature in the dark with calcium-containing annexin V-binding buffer (10-mmol/L N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid and sodium hydroxide, pH7.4, 140-mmol/L sodium chloride, 2.5-mmol/L calcium chloride) and analyzed with an Ortho Cytron Absolute. When measuring P-selectin, PAC-1, and annexin V, which were present at low levels on unstimulated platelets and appear at significant levels on activation, the data were expressed as the percentage of fluorescence-positive platelets.

Platelet-derived microparticles (PMPs) were detected with a modification of a documented method.²⁰ Whole blood was incubated with 10 μ L SZ22 (platelet identifier monoclonal antibody) for 30 minutes at room temperature in the dark. Only particles positive for GpIIb were gated, to distinguish PMPs from electronic noise. A sample of 10,000 FITC-positive particles in the PMP gate was then counted to determine the number of PMPs released per 10,000 platelets.

Statistical Analysis

StatView 5.0 (SAS Institute, Inc, Cary, NC) was used for statistical analysis. All data are given as mean $\pm$ SD. Differences at each sampling of data between the two groups were assessed with the Student t test. Comparisons between groups were made with 2-factor repeated measures analysis of variance. When the analysis of variance indicated a significant effect, the differences between the two groups were evaluated with the Student t test. The Pearson correlation coefficient was determined for the analysis of the relationship between platelet number and each platelet activation marker.

Results

Prevention of Platelet Function During in Vitro Extracorporeal Circulation by Various Platelet Inhibitors

When heparinized blood was recirculated with vehicle only (control, n = 5), platelet counts (initial count $10.98 \pm 1.80 \times 10^4$ cells/ μ L) gradually fell to $58.2\% \pm 6.2\%$ of the initial level at 180 minutes, as shown in Figure 1 and Table

TABLE 1. Dose-dependent effects of FK633, abciximab, and PGE₁ on platelet activation during in vitro extracorporeal circulation

	Control	FK633		Abciximab		PGE ₁	
		100 nmol/L	500 nmol/L	2 µg/mL	10 µg/mL	50 ng/mL	100 ng/mL
Platelet counts (10 ⁴ cells/mm ³)							
Before	11.0 ± 1.8	10.3 ± 0.9	10.2 ± 1.1	10.3 ± 2.0	10.7 ± 2.1	11.7 ± 1.3	11.9 ± 1.6
180 min	6.3 ± 1.0	7.7 ± 1.0*	8.6 ± 0.8†	9.5 ± 1.9‡	10.1 ± 2.2‡	7.9 ± 0.6*	9.0 ± 0.7‡
GpIb (mean fluorescence intensity)							
Before	148.9 ± 2.9	149.9 ± 3.6	149.5 ± 3.7	148.8 ± 2.6	148.9 ± 2.0	148.0 ± 2.9	148.2 ± 2.9
180 min	130.2 ± 3.8	130.4 ± 3.2	135.3 ± 4.5*	134.7 ± 3.1*	135.8 ± 4.0*	132.3 ± 5.9	137.4 ± 5.1*
GpIIb (mean fluorescence intensity)							
Before	168.0 ± 4.2	165.4 ± 3.2	168.3 ± 2.9	169.0 ± 2.7	169.6 ± 0.8	167.7 ± 2.9	167.3 ± 2.9
180 min	161.6 ± 1.5	161.8 ± 4.6	162.8 ± 2.0	164.1 ± 2.0	165.2 ± 1.7	160.0 ± 5.0	161.2 ± 2.5
PAC-1 (% positive platelets)							
Before	4.6 ± 1.2	4.7 ± 0.8	4.5 ± 1.5	1.2 ± 0.2	1.2 ± 0.4	3.5 ± 1.5	3.3 ± 1.4
180 min	27.1 ± 5.9	26.4 ± 7.5	8.7 ± 2.0‡	1.5 ± 0.7‡	1.6 ± 0.4‡	13.1 ± 4.0†	6.8 ± 2.8‡
P-selectin (% positive platelets)							
Before	4.0 ± 0.7	4.6 ± 1.5	4.7 ± 1.5	3.6 ± 1.0	3.5 ± 1.3	4.5 ± 0.3	4.3 ± 0.3
180 min	14.6 ± 1.9	11.4 ± 2.8†	11.7 ± 2.5†	9.9 ± 1.6‡	9.5 ± 2.5‡	11.3 ± 2.6†	8.1 ± 1.4‡
Annexin V (% positive platelets)							
Before	3.5 ± 0.8	3.6 ± 1.3	3.6 ± 1.0	2.3 ± 1.0	2.3 ± 1.0	5.4 ± 2.4	5.1 ± 1.7
180 min	36.6 ± 4.1	16.8 ± 4.3†	14.1 ± 4.5†	19.1 ± 4.6†	14.5 ± 5.7†	21.0 ± 7.3*	9.4 ± 1.9‡
PMPs (per 10 ⁴ platelets)							
Before	227 ± 81	239 ± 38	221 ± 38	180 ± 38	199 ± 45	245 ± 84	233 ± 56
180 min	1241 ± 250	1136 ± 358	928 ± 182*	737 ± 227†	466 ± 217†	940 ± 393*	692 ± 167†

Platelet counts; platelet surface expressions of GpIb, GpIIb, PAC-1, P-selectin, and annexin V, and PMP counts in the control group and groups with antiplatelet drugs FK633 (100 and 500 nmol/L), abciximab (2 and 10 µg/mL), and PGE₁ (50 and 100 ng/mL) before in vitro extracorporeal circulation and 180 minutes afterward. All data are shown as mean ± SD (n = 5).

**P* < .05 between the control group and the group given antiplatelet drugs.

†*P* < .01 between the control group and the group given antiplatelet drugs.

‡*P* < .001 between the control group and the group given antiplatelet drugs.

1. These results were similar to those reported previously for recirculated blood without platelet inhibitors.^{1,2} There was no significant change in the platelet surface expression of GpIIb throughout the circulation (Table 1). The percentage of PAC-1 (activated GpIIb/IIIa complex)-positive platelets increased from 4.6% ± 1.2% before circulation to 8.6% ± 3.1% after 5 minutes of extracorporeal circulation in the control circuits and reached the peak (27.1% ± 5.9%) after 180 minutes of circulation. The percentage of annexin V-positive platelets (surface expression of phosphatidylserine) increased from 3.5% ± 0.8% at initial level to 36.6% ± 4.1% after 180 minutes of circulation in the control group (Table 1). The expression of surface GpIb (mean fluorescence intensity) in the control group (148.9 ± 2.9 at the initial level) decreased significantly during circulation, reaching the lowest level (130.2 ± 3.8) after 180 minutes of extracorporeal circulation, as reported previously elsewhere.⁶ The expression (percentage of positive platelets) of platelet surface P-selectin (the integral membrane protein of α-granule) increased from 4.0% ± 0.7% before circulation to 8.9% ± 0.9% after 5 minutes of extracorporeal circulation and reached the peak (14.6% ± 1.9%) after 180 minutes of circulation. During extracorporeal circulation the release of PMPs was increased from 227 ± 81 per 10⁴

platelets before circulation to 1241 ± 250 per 10⁴ platelets after 180 minutes of circulation.

GpIIb/IIIa complex antagonists FK633 (500 nmol/L) and abciximab (10 µg/mL) significantly inhibited the decrease in platelet counts induced by in vitro CPB circulation (Figure 1). These inhibitors dose dependently prevented platelet loss during heparinized CPB circulation (Table 1). PGE₁ at 50 ng/mL also had a protective effect on platelet number, but the protective effect of PGE₁ was less. Abciximab, FK633, and PGE₁ blocked increases in PAC-1-positive platelets, annexin V binding, expression of P-selectin, and PMP formation during simulated CPB circulation (Figure 1 and Table 1). Among these drugs, abciximab appeared to be the most effective. Although abciximab completely blocked platelet loss and the increase in PAC-1-positive platelets, the antibody only attenuated expression of other functional markers such as P-selectin, annexin V binding, and PMP formation. We then analyzed the relationship between platelet counts and functional markers of platelet activation. The correlation coefficients between platelet number and expressions of PAC-1, annexin V, GpIb, P-selectin, and PMP generation were −0.622 (*P* < .0001), −0.527 (*P* < .0001), 0.476 (*P* = .0002), −0.557 (*P* < .0001) and −0.477 (*P* < .0001), respectively. Figure 2 shows data on the negative

Figure 2. Correlation of platelet numbers and PAC-1 or P-selectin expressions during simulated CPB circulation.

correlation between platelet numbers and expressions of PAC-1 and P-selectin. PAC-1 expression was most strongly correlated with the decrease in platelet count during simulated CPB circulation.

An anti-human vWF monoclonal antibody, AJvW-2, and a polymeric anionic aromatic compound, ATA, both of which inhibit vWF binding to platelet GpIb, did not prevent the decrease in thrombocyte counts during simulated CPB circulation, as shown in Figure 3. These inhibitors did not inhibit increases in PAC-1 expression, annexin V binding, P-selectin expression, and PMP formation during *in vitro* extracorporeal circulation.

Combined Effects of Argatroban and FK633 on Platelet Activation During Extracorporeal Circulation

Because thrombin is formed and circulates during heparinized extracorporeal circulation,⁵ platelets probably are activated even though aggregation and adhesion are inhibited. Argatroban, a small-molecule synthetic direct thrombin inhibitor, binds rapidly and reversibly to both clot-bound and soluble thrombin.¹⁴ The relatively short elimination half-life of argatroban (40-50 minutes) and its reversible binding allow rapid achievement of therapeutic effects on initiation of therapy and rapid restoration of normal hemostasis on cessation of therapy. We tested argatroban in an *in vitro* extracorporeal circulation as a substitute for heparin.

As shown in Figure 4, argatroban alone better maintained the platelet number, and there was a lesser degree of increase in the expression of P-selectin than seen with heparin alone. In the group treated with FK-633 and argatroban, platelet counts at 180 minutes were significantly higher than those in the group given FK-633 and heparin. In the group given FK-633 and argatroban, expressions of PAC-1 and P-selectin at 180 minutes of extracorporeal circulation were also significantly more inhibited than seen in the group given FK-633 and heparin. However, there were no significant differences in annexin V binding and PMP formation between these two groups.

Discussion

Our study indicates that the decrease in platelet number was most strongly correlated with increase in the expression of PAC-1 during extracorporeal circulation with heparin, although other platelet activation markers tested also had negative correlations with platelet number. Gradual platelet consumption is thought to be the result of platelet adhesion and activation to foreign surfaces in the extracorporeal circuits,² partly mediated by interaction of platelet GpIIb/IIIa complex receptors and fibrinogen bound to the surfaces of bypass tubing.²¹ It seems reasonable to assume that the direct inhibition of interaction between active GpIIb/IIIa complex receptor and fibrinogen effectively contributes to preservation of platelet counts in CPB circulation. GpIIb/IIIa complex antagonists abciximab and FK633 prevented platelet loss almost completely during simulated CPB circulation, whereas these drugs only attenuated platelet activation, as indicated by the expression of P-selectin (α -granule secretion) and annexin V binding (procoagulant activity). Although PGE₁ inhibited platelet activation and preserved platelet function, the concentration of PGE₁ was higher than noted in clinical use.²² In addition, PGE₁ is a potent vasodilator that is rapidly metabolized in the pulmonary circulation, and therefore its clinical use could be limited.²³ ATA, with a molecular mass of less than 5 kd, inhibited vWF-mediated platelet aggregation.¹¹ Borgdorff and colleagues²⁴ reported that ATA inhibited pump-induced platelet aggregation and the loss of platelets in an extracorporeal circuit under shear stress, which was higher than in our experimental condition. AJvW-2 (an anti-vWF monoclonal antibody) inhibited vWF binding to platelet GpIb by ristocetin and blocked vWF-GpIb interaction under conditions of a high shear rate.¹⁰ ATA and AJvW-2, inhibitors of interaction between vWF and GpIb, had no protective effect against platelet loss under our flow conditions with low shear stress (approximately 30 dyne/cm²). Temporary and reversible pharmacologic inhibition of platelet activation may be needed during CPB to reduce postoperative bleeding in cardiac surgery. Abciximab more effectively preserved platelet counts and function than did FK633 during

Figure 3. Effects of AJvW-2 and ATA on platelet counts; expressions of platelet surface GpIb, PAC-1, P-selectin, and annexin V; and PMP formation during simulated CPB circulation with heparin as anticoagulant. Heparinized (3 U/mL) blood was recirculated with vehicle (control, circles), 200 µg/mL ATA (diamonds), or 3.0 µg/mL AJvW-2 (inverted triangles). Data points represent mean; error bars represent SD (n = 5).

extracorporeal circulation. Abciximab is a Fab fragment of chimeric mouse/human immunoglobulin G antibody. Its plasma half-life is 26 minutes, but the antiplatelet effect is prolonged and irreversible after administration. Elimination of abciximab from the human body in a clinical setting is considerably slower; the catabolic beta half-life was estimated to be 7 hours.^{8,9} Preoperative treatment with abciximab does not increase surgical mortality, but exposure before an operation has been reported to increase postoperative bleeding and transfusion requirements and is associated with a higher incidence of mediastinal reentry.²⁵ Currently it is impossible to neutralize the action of abciximab at the end of extracorporeal circulation. Reversible GpIIb/IIIa complex inhibitors tirofiban⁶ and eptifibatide²⁶ have shown to reduce platelet activation during CPB and to decrease postoperative bleeding time in baboons. FK633 is an arginine-glycine-aspartic acid peptide-mimetic GpIIb/IIIa complex antagonist with a reversible antiplatelet effect and its plasma half-life is 0.52 hours,⁷ which is shorter than those of tirofiban and eptifibatide. Therefore a low-molecular weight GpIIb/IIIa complex antagonist such as FK633

may be a potential candidate for platelet protection during CPB, although FK633, even at high doses, is less active against platelet loss and PAC-1 expression during an in vitro CPB circulation than is abciximab.

We have reported that the thrombin-antithrombin complex, a marker of thrombin generation, increases progressively and significantly during in vitro CPB circulation, even if heparinized platelets are recirculated, and then decreases after termination of pumping.⁵ In addition to contact activation of platelets, thrombin production may also contribute to activating platelets during simulated CPB, the result being increases in P-selectin expression, PAC-1 expression, and PMP formation. Heparin enhances platelet aggregation induced by agonists such as adenosine diphosphate and epinephrine^{27,28} and shear stress-induced platelet aggregation at both low and high shear stresses,²⁹ whereas heparin inhibits platelet reaction resulting from the action of thrombin.³⁰ Treatment with argatroban, a direct inhibitor of thrombin with a short half-life, prevented platelet loss and elevation of P-selectin more effectively than did heparin during a simulated CPB circulation. There appeared to be no

Figure 4. Combined effects of FK633 and argatroban on platelet counts; expressions of platelet surface GpIb, PAC-1, P-selectin, and annexin V; and PMP formation before circulation and at 180 minutes of in vitro extracorporeal circulation. Blood was recirculated with heparin (3 U/mL) alone (filled circles), heparin (3 U/mL) plus FK633 (500 nmol/L, open circles), argatroban (3 μ g/mL) alone (filled triangles), or argatroban (3 μ g/mL) plus FK633 (500 nmol/L, open triangles). Data points represent mean; error bars represent SD (n = 5). Plus signs indicate $P < .05$ and asterisks indicate $P < .01$.

significant differences as to annexin V binding and PMP formation between these groups. The combination of argatroban and FK633 significantly inhibited platelet loss and expressions of PAC-1 and P-selectin relative to findings with heparin and FK633. Despite full inhibition of PAC-1 expression, the combination of both inhibitors only partially prevented annexin V binding and PMP formation. Our findings suggest that inhibition of both thrombin and platelets is effective to preserve platelet number and function during CPB circulation. For cardiac operations, prompt and complete reversal of platelet inhibition is an absolute requirement. The clinical disadvantage of argatroban is that no neutralizing agent exists, such as protamine for heparin. Therefore these combined therapies still may not be sufficient to produce a meaningful decrease in postoperative bleeding after cardiovascular operation. Definitive assessments of efficacy and safety of combination of FK633 and argatroban await in vitro and in vivo studies. It has become increasingly evident that potent but reversible inhibition of both of platelet aggregation and thrombin activity will be

needed if optimal extracorporeal circulation without platelet loss is to be achieved.

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