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A Randomized Clinical Trial Investigating the Relationship Between Aprotinin and Hypercoagulability in Off-Pump Coronary Surgery

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Abstract

BACKGROUND—Off-pump coronary artery bypass (OPCAB) surgery is associated with a hypercoagulable state in which the platelet thrombin receptor, protease-activated receptor-1 (PAR-1), helps propagate a thrombin burst within saphenous vein grafts. Aprotinin, used in cardiothoracic surgery mainly for its antifibrinolytic properties, also spares platelet PAR-1 activation due to thrombin. We hypothesized that this PAR-1 antagonistic property provides an antithrombotic benefit during OPCAB surgery.

METHODS—Patients were randomly assigned to receive saline (n = 38) or a modified full-dose regimen of aprotinin (n = 37) IV during OPCAB surgery. Blood sampled perioperatively from the coronary sinus, skin wounds, and systemic circulation was analyzed to test coagulation and platelet function. Major adverse cardiovascular events were monitored by obtaining troponin I at 24 h (myocardial infarction), predischarge computed tomography angiography (vein graft thrombosis), and by clinical examination for stroke.

RESULTS—Coronary sinus blood obtained immediately after OPCAB surgery showed significantly less activation in the aprotinin group, as judged by reduced formation of platelet-leukocyte conjugates ($P < 0.02$) and platelet-derived microparticles ($P < 0.05$). The aprotinin group showed inhibition of platelet aggregation induced by thrombin ($P = 0.007$) but not adenosine diphosphate. Thrombin generation, defined by F1.2 levels, was significantly reduced by aprotinin in the coronary sinus but not in skin wound incisions. Major adverse cardiovascular events were significantly reduced in aprotinin-treated patients (5.4% vs 29.7%, $P < 0.05$). Aprotinin also demonstrated antifibrinolytic properties through diminished red blood cell transfusion ($P < 0.04$) and reduced blood loss postoperatively (603 ± 330 vs 810 ± 415 mL, $P < 0.004$).

CONCLUSION—This study demonstrates that aprotinin protects patients undergoing OPCAB surgery from a hypercoagulable state by diminishing thrombin-induced platelet activation and thrombin generation within saphenous vein grafts, while maintaining systemic hemostatic and

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antifibrinolytic benefits. These results support further investigation of aprotinin and other PAR-1 antagonists in OPCAB surgery.

Thrombin is the central enzymatic mediator of both hemostasis and thrombosis. Thrombin produced in a controlled fashion creates fibrin polymers critical for hemostasis while a larger burst activates platelets via its high affinity thrombin receptor, protease-activated receptor-1 (PAR-1), leading to a platelet-rich clot and thrombosis.[1] Blood collected from standardized bleeding time skin incision on the forearm provides a model of hemostasis in response to microvascular injury.[2,3] Because surgically grafted vessels always develop some degree of perioperative endothelial disruption,[4] they provide a "model" of macrovascular injury. Blood sampled from the coronary sinus (CS) downstream of these grafts provides a unique opportunity to assay the regional thrombotic response to this injury.[5] We have documented a significant increase in regional thrombin production downstream of saphenous vein grafts (SVGs) after off-pump as compared with on-pump coronary artery bypass graft (CABG),[6] with the level of thrombin produced directly related to the risk of early SVG failure.[5] This heightened regional thrombin production is particularly noteworthy in light of recent meta-analyses demonstrating that the risk of early SVG failure is increased after off-pump coronary artery bypass (OPCAB) grafting.[7,8]

PAR-1 receptor antagonists have demonstrated the ability to target the cellular actions of thrombin on platelets while leaving the hemostatic actions of thrombin in the coagulation cascade untouched.[9] The hemostatic agent, aprotinin, has been shown to be an antagonist of PAR-1 at pharmacologically relevant doses.[10,11] This inhibitory effect would be predicted to provide clinical antithrombotic actions on platelets within the macrovasculature without hindering hemostatic plug formation in the microvasculature at wound sites where alternate platelet agonists (e.g., collagen and adenosine diphosphate [ADP]) are generated.[12] On the other hand, aprotinin also inhibits fibrinolysis, which raises the concern that it might increase the risk of thrombosis. Although the link between aprotinin and thrombosis has endured "twenty-five years of claim and counterclaim,"[13] there have been essentially no clinical studies on the mechanism of how aprotinin may cause thrombosis. The purpose of the study was to test the hypothesis that aprotinin administration would inhibit the pathologic burst in thrombin production produced within grafted macrovessels but not physiologic thrombin required for wound hemostasis.

METHODS

Patient Selection and Enrollment

A randomized, double-blind, placebo-controlled study of aprotinin was completed in 75 OPCAB patients recruited between August 2005 and January 2007 (IRB protocol #0902312). Because the use of aprotinin for OPCAB is "off-label," a Food and Drug Administration Investigational New Drug application was submitted and approved (IND #67,890). Exclusion criteria included patients with creatinine >2.0 mg/dL, active hepatitis or cirrhosis, allergy to radiographic contrast media, prior aprotinin use, and clopidogrel use within 3 days of surgery. Randomization was based on permuted blocks of Size 4 to preserve ongoing balance between groups. Patient demographics and perioperative data were gathered prospectively and recorded onto case report forms. Laboratory results were captured through an interface with the university's Clinical Data Repository.

Treatments

A modified "full-dose" regimen was used[14]: 10,000 kallikrein inhibiting units (KIU) IV test dose (or saline placebo), 2 million KIU via a central line, and 500,000 KIU/h IV until the end of surgery. Heparin was given at a dose calculated to obtain a kaolin-based activated clotting

time >300 s with further heparin given to maintain a level >2 U/mL. The heparin effect was partially reversed by half the recommended dose of protamine. Pre- and postoperative aspirin (325 mg PO/d) was the sole platelet inhibitor used in all patients. Transfusions were based on a previously described algorithm.[14] Intensive care unit and hospital discharge followed established protocols. All clinicians, including the single surgeon who performed all OPCAB operations, were blinded to treatment group.

Surgery

OPCAB surgery was performed by a single surgeon. Internal mammary conduits were used in all patients. Conduit harvest was performed with an open technique for the radial artery and endoscopically (VasoView6, Guidant Systems, Minneapolis, MN) for the saphenous vein. Distal anastomoses were facilitated with suction-based devices. Conversion to a standard, on-pump CAB technique ($n = 2$) resulted in exclusion from analysis per protocol. The volume of shed blood was recorded intraoperatively using a Cell Saver device and at 1 and 24 h using the chest drain. Patients were excluded from the study per protocol if the findings at reexploration for bleeding revealed a “surgical” source according to the operative surgeon ($n = 1$).

Conduit Quality Assessment

Flow—Blood flow and pulsatility index were assessed in each completed bypass conduit using a transit-time flow meter (Medistim, Minneapolis, MN). SVGs with flow that remained <10 mL/min and pulsatility index >5 despite revision were excluded from the patency analysis ($n = 2$) per protocol.

Intimal Integrity—Before grafting, SVGs were imaged intraoperatively with optical coherence tomography (ImageWire, LightLab Imaging, Westford, MA) and retained clot or intimal injury within the SVG quantified as previously described.[15] Luminal tissue factor activity was determined in SVG segments isolated in a custom-designed chamber.[16] A reaction buffer was added containing factors VII (2 U/mL, American Diagnostica, Greenwich, CT) and X (2 U/mL, American Diagnostica) with EDTA used to stop the reaction. The generation of factor Xa was measured in the supernatant by adding a chromogenic substrate (Spectrozyme FXa, American Diagnostica) and the absorption measured at 405 nm.

Blood Collection—Blood samples were obtained preoperatively before skin incision, postoperatively 30 min after protamine administration (systemically and from the CS via direct puncture using an 18-gauge needle), and Days 1 and 3. Blood emanating from a standardized skin wound created on the forearm using a commercially available device (Simplate II, Organon Teknika) was collected into heparinized plastic tubes. Citrated (3.8%) blood was analyzed for cardiac troponin I (CTnI) levels by enzyme-linked immunosorbent assay (Genwaybio) and for coagulation and platelet function as follows.

Thrombin Formation and Activity—After the addition of D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK, Sigma), platelet poor plasma was obtained by centrifugation at 2000*g* and was then analyzed for F1.2 and fibrinopeptide A using enzyme-linked immunosorbent assay kits (Enzygnost F1.2 micro, Dade-Behring, and fibrinopeptide A, Boehringer). The transmyocardial gradient in these markers was analyzed from simultaneously procured blood samples from the aorta (Ao) and CS using the equation: ([CS – Ao]/CS) × 100.

Platelet Function Testing—Whole blood aggregometry (Chronolog, Hawerton, PA) was performed using previously described techniques[14] and impedance change ([Omega]) was assessed in whole blood at 6 min after addition of a series of agonists: 1) thrombin (0.25, 0.5, 0.75, 1.0 U/mL), 2) ADP (5, 10 mM), and 3) collagen (1, 5 μg/mL). Thrombelastography was

performed by the addition of calcium chloride (2 mM) and tissue factor (20 μM). Flow cytometry (Becton Dickinson) was performed on whole blood diluted with modified Tyrode buffer and 5 mM gly-pro-arg-pro (GPRP, Centerchem). Samples fixed with paraformaldehyde were analyzed by fluorescence-activated cell sorter (Becton Dickinson) with platelets identified by its constitutive receptor, CD41a (Fig. 1A), and microparticles defined as annexin V^+, CD41a+ particles <1 μm diameter (Fig. 1B). Leukocyte-conjugated platelets were identified by forward scatter to identify a CD41a+ and annexin V^+ subpopulation of larger size than single platelets (Fig. 1C). Both were normalized against the number of CD41a+ platelets in the sample.[17]

Postoperative Follow-Up

Stroke was identified by daily physical examinations and confirmed by head computerized tomography (CT) examination. Noninvasive, 64-detector row, chest CT angiography (420 ms rotation, 100–150 mL contrast agent IV at 5 mL/s, retrospective electrocardiographic gating) was obtained to assess patency of SVGs by a single, blinded, expert reviewer. SVGs were defined as "patent" if any flow was seen through the graft regardless of the presence of stenosis and "non-patent" if a stump was seen or if no flow was observed by CT angiography. Postoperative myocardial infarction (MI) was defined by an increase in postoperative CTnI level that was 5 times the upper limit of normal or an electrocardiogram showing new Q waves at 4, 12, or 72 h after surgery.

Statistical Methods

The primary end point was a comparison of regional hypercoagulability between groups as defined by the transcardiac gradients of F1.2, FPA, and platelet-derived MPs. Preliminary data showed a threefold reduction in the transcardiac F1.2 gradient for aprotinin versus placebo.[14] Power analysis indicated that 35 patients per group were required to confirm this difference in the gradients between groups at 2-sided $P = 0.05$ and power = 80% (http://hedwig.mgh.harvard.edu/sample_size/size. html#parallel). The composite of arterial thrombotic events during the first postoperative week (early SVG failure, MI, and stroke) was a secondary clinical end point. Unless specified, comparisons were done by analysis of variance with subsequent pairwise comparisons according to the Duncan multiple range test. Categorical data were compared using Fisher's exact test. A P value <0.05 was considered statistically significant. Statistical analysis was performed using the InStat™ statistical package with the assistance of a statistician. The sponsors of this trial assisted in study design but played no role in the collection, analysis, and interpretation of data.

RESULTS

Study Population

During the enrollment period, 693 patients were screened and 75 completed the full protocol, with 38 receiving aprotinin and 37 receiving placebo. Patients were excluded due to primary on-pump CABG ($n = 390$) or intraoperative conversion ($n = 2$), creatinine >2.0 mg/dL preoperatively ($n = 65$) or postoperatively ($n = 3$, 2 from the aprotinin group and 1 from the placebo group), inability to obtain informed consent ($n = 100$), preoperative use of antiplatelet drugs other than aspirin ($n = 57$), or postoperative bleeding due to a surgical source ($n = 1$). There were 2 perioperative deaths, 1 in the aprotinin group and 1 in the placebo group and no episodes of hypersensitivity reactions or renal failure requiring dialysis.

Two hundred twenty-seven grafts were placed with no difference in the average number of grafts/patient between groups (3.1 vs 3.2). No differences were noted between the aprotinin and placebo groups in the patient demographics or preoperative risk factors and medications.

Intraoperatively collected data such as ejection fraction, conduit diameter, target size and quality, and inotropic requirements were also similar.

Bleeding and Transfusions

Aprotinin significantly reduced bleeding intraoperatively, over the first postoperative hour and first 24 h (Table 1). Two patients in the placebo group and 1 in the aprotinin group required postoperative reexploration for bleeding. The aprotinin group showed significantly reduced red blood cell transfusion requirement (Table 1) with 31% of aprotinin patients requiring transfusion compared with 44% of the placebo group ($P = 0.18$). Additionally, aprotinin treatment was associated with fewer transfusions of fresh frozen plasma (0.0% vs 3.8% of patients) and platelets (4.1% vs 6.9%), although the differences did not reach statistical significance.

Coagulation and Platelet Function

No differences between groups were seen in international normalized ration, activated partial thromboplastin time, platelet count, D-dimer level, or fibrinogen at any time point. Both groups showed a postoperative decrease in the maximum amplitude of the TEG® tracing with no significant difference noted between groups (6.18% ± 6.16% vs 4.17% ± 5.28% decrease in maximum amplitude compared with preoperatively, P = not significant [NS]). Immediately postoperatively, the aprotinin group showed a significant reduction in *ex vivo* platelet aggregation in response to thrombin 1 U/mL (74.4% ± 0.22% reduction in impedance compared with baseline, $P < 0.0001$, paired *t*-test). The placebo group also showed a decrease in the thrombin aggregation response (Fig. 2A) but was significantly more responsive to thrombin than the aprotinin group (Fig. 2B, Table 2). No significant differences were seen between groups in the postoperative aggregation response to 2 concentrations of collagen (1 and 5 μg/mL) and ADP (5 and 10 mM) (Table 2).Thrombin generation after microvascular injury, defined by the F1.2 level in blood emanating from a standardized incisional skin wound, was not significantly different between groups (Fig. 3, Table 2).

Thrombotic Events

Early attrition occurred in 0 internal mammary artery grafts, 1 radial artery graft, and 7 SVGs during the study, which included 6 of 46 SVGs (13.0%) from the placebo group and 1 of 44 SVGs (2.3%) from the aprotinin group ($P = 0.056$). The combined incidence of postoperative SVG failure (1 vs 6), MI (1 vs 4), and stroke (0 vs 1, aprotinin versus placebo, respectively) was significantly reduced in the aprotinin group compared with placebo (5.4% vs 29.7%, $P < 0.05$). CTnI levels on the first postoperative day were significantly reduced for the aprotinin versus placebo group (1.72 ± 1.44 vs 5.14 ± 7.23 ng/mL, $P = 0.01$).

Compared with those free of adverse events, patients who developed a thrombotic event showed no difference in the amount of bleeding measured either intraoperatively (908 ± 527 vs 990 ± 481 mL, P = NS) or postoperatively (585 ± 303 vs 605 ± 351 mL/24 h, P = NS).

Risk for SVG Thrombosis

Intraoperative blood flow within the SVG, as determined by ultrasonic flow measurements, was not significantly different between groups (61.38 ± 29.7 vs 55.97 ± 29.6 mL/min, P = NS), suggesting that target runoff and anastomotic quality were similar between groups. The quality of the conduits was also similar with all SVGs showing at least some retained clot (20.7% ± 26.3% vs 32.5% ± 30.1% of the conduit length with evidence of clot), denuded endothelium (74.2% ± 14.9% vs 78.0% ± 20.9% endothelial integrity), and upregulated tissue factor activity (1.37 ± 0.6 vs 1.07 ± 0.6 U/cm^2). In contrast, internal mammary artery grafts showed no retained

clot, near perfect endothelial integrity (94.5% ± 3.5% vs 96.0% ± 3.1% integrity), and less tissue factor activity (0.6 ± 0.3 vs 0.5 ± 0.4 U/cm^2).

Subjects in both groups received at least 1 SVG (1.17 ± 0.64 vs 1.24 ± 0.89 venous grafts/patient, *P* = NS). Given the thrombogenicity of these conduits, the analysis of CS blood provided an appropriate means to compare the regional thrombotic response with macrovascular (i.e., SVG) injury. We noted an increase in the transcardiac (i.e., CS versus arterial) gradient of F1.2 levels measured 30 min after protamine administration in both groups (Table 3). However, patients receiving aprotinin compared with placebo had significantly reduced gradients of F1.2 but no difference in fibrinopeptide A, a marker of fibrin formation (Fig. 4, Table 3). Platelet-derived MPs and platelet-leukocyte conjugates were also significantly reduced in the aprotinin group compared with placebo (Table 3).

DISCUSSION

We present evidence that aprotinin can inhibit platelet activation and thrombin generation within SVGs of patients undergoing OPCAB surgery, while simultaneously maintaining hemostatic responses in incisional wounds and clinical transfusion benefits. Such apparently contradictory properties are explained through the ability of aprotinin to target pathways mediating platelet PAR-1 activation and fibrinolysis, while sparing enzymatically mediated thrombin production required for hemostasis.

Before its withdrawal from the market in 2008, aprotinin was used primarily as an antifibrinolytic drug—a property mediated through its inhibition of plasmin.[18] However, aprotinin targets a wide range of other serine proteases that are activated during cardiac surgery.[18] The thrombin receptor PAR-1 is a cell-based target that is dose-dependently inhibited by aprotinin. *In vitro* studies have shown that aprotinin inhibits thrombin-induced platelet activation, while leaving nonproteolytic pathways of activation (i.e., via collagen or ADP) unaffected.[19] Subsequent clinical studies have been consistent with predictions from the *in vitro* studies,[10,20] but a stronger validation of the PAR-1 antiplatelet mechanism of action in the clinic has now been addressed in this study in off-pump surgery.

OPCAB presents an ideal model for investigating the thrombin burst and thrombotic complications within SVGs. Surgery on the beating heart is linked to a hypercoagulable state in which heightened platelet PAR-1 receptor activation participates in a feedback loop of thrombin generation unencumbered by the coagulopathy associated with cardiopulmonary bypass.[5,6] An important design element of our study was to contrast pathologic thrombin generation in the CS[6] with physiologic thrombin generation in a skin incision model of hemostasis.[3] Despite attempts to minimize procurement-related trauma during this study, all SVGs were at least partially denuded of endothelium during harvest, thus increasing local tissue factor activity and presenting SVG as a “model” of macrovascular injury. Because of the clinical intrusiveness of the protocol, CS measurements were limited to a single 30-min time point. Preliminary data on the kinetics of regional thrombin formation after OPCAB surgery suggest its rapid release from the bypass grafts within minutes of protamine administration, reminiscent of the well-described “rebound hypercoagulable state” in patients with unstable angina after heparin withdrawal.[21,22] The subendothelium of the injured SVG provides a fertile environment for a rebound hypercoagulability that increases the risk of acute SVG failure.[5] The fact that F1.2 levels in OPCAB patients were inhibited by aprotinin in CS blood but not in the bleeding time model is fully consistent with selective targeting of thrombin's actions on cellular but not enzymatic targets. The additional reduction of platelet MPs and leukocyte conjugates in CS blood suggests the interruption of a pathway that is likely to be pathophysiologically important to the newly grafted SVG.

Whole blood aggregation performed *ex vivo* in CS blood confirmed that the antiplatelet actions of aprotinin were selective for thrombin-induced platelet activation with no effect on other platelet agonists, again consistent with targeting of PAR-1. Aprotinin therefore exhibits actions similar to PAR-1 peptidomimetic antagonists undergoing clinical development for use in cardiac surgery, which have demonstrated a reduction in major adverse cardiovascular events via targeting the platelet thrombin receptor without causing increased bleeding.[23] These actions of aprotinin are distinct from other methods of inhibiting platelet activation, such as aspirin, which improves SVG patency after CABG but also incurs a bleeding risk through decreased thrombin production in skin wounds.[3] Familiarity with the bleeding risk from standard antiplatelet therapy is often extrapolated to mean that bleeding reduces the risk of thrombotic events, such as SVG failure. This belief, in turn, creates anxiety about reducing blood loss through the use of hemostatic drugs. We found no relationship between the rate of perioperative bleeding and whether the patient developed a thrombotic event. Our analysis of thrombin production within the SVG compared with the skin wound provides new mechanistic insight into the clinical relationship between hemostasis, thrombosis, and antifibrinolytics. The central point arising from this investigation is that a hemostatic drug need not carry with it an inevitable prothrombotic risk.

Our proposal that aprotinin has antithrombotic mechanisms is contradicted by other reports. A recent, large randomized trial, the BART Study,[24] as well as retrospective reports,[25,26] suggest that aprotinin use increases mortality after cardiac surgery. An important difference from our study is the use of cardiopulmonary bypass. In fact, the BART study enrolled only the highest risk patients associated with cardiopulmonary bypass times that often exceeded 2 h. These extended times promote the cleavage and downregulation of the platelet PAR-1 receptor and eventually lead to a phenomenon called “platelet exhaustion.”[27] We have found that OPCAB maintains PAR-1 receptor integrity, allowing platelets to participate in events linked to hypercoagulability,[5] such as heightened regional thrombin production, compared with on-pump CABG.[6] Differences in the quantity of PAR-1 available for antagonism could explain the pharmacodynamic differences seen for aprotinin use during on-pump versus off-pump CABG procedures.

The main limitation to this study was the small size, which required the use of a composite end point rather than a more unambiguous primary end point for defining the effect of aprotinin on hypercoagulability such as SVG patency. The strong trend toward significantly less early graft closure in the aprotinin group was noteworthy given that each SVG was screened for abnormalities at the level of the graft that might influence patency that are not due to the effects of aprotinin and hypercoagulability (e.g., conduit blood flow and intimal quality). Combined with a reduction in prothrombotic markers, our study suggests that it is very unlikely that aprotinin exacerbates hypercoagulability after OPCAB surgery. However, the challenges of deciphering the clinical effects of a drug with multiple targets such as aprotinin limit our ability to provide more definitive conclusions about mechanisms. For example, we noted fibrin formation within SVGs soon after heparin reversal (i.e., increased transcardiac gradient of fibrinopeptide A), raising concerns about the capacity for a compensatory fibrinolysis after aprotinin use. In addition, the mode of activation of the coagulation system in the skin wound incision is likely very different than in the bypass grafts, which complicates our efforts to compare assays obtained from these 2 compartments. The net effect of aprotinin on SVG thrombosis after OPCAB surgery can only be determined by an appropriately designed trial to establish the mechanistic link between these regional biochemical events and clinical graft patency. Finally, an analysis of other potential safety concerns of aprotinin use during OPCAB surgery unrelated to thrombosis (e.g., renal failure) has been published[28] but was beyond the scope of the current study.

In conclusion, this study presents evidence that aprotinin can exert antithrombotic effects in SVG after OPCAB surgery while maintaining controlled thrombin production in hemostasis and clinical antifibrinolytic benefits. Such apparently contradictory properties can be understood, and indeed have been predicted, based on multiple targeting of the thrombin receptor PAR-1 on platelets and plasmin in the fibrinolytic pathway. Better understanding of the clinical mechanisms of action of aprotinin will inform the debate on the safety of aprotinin and spur the investigation of successor compounds to aprotinin and novel PAR-1 antagonists for cardiac surgery.

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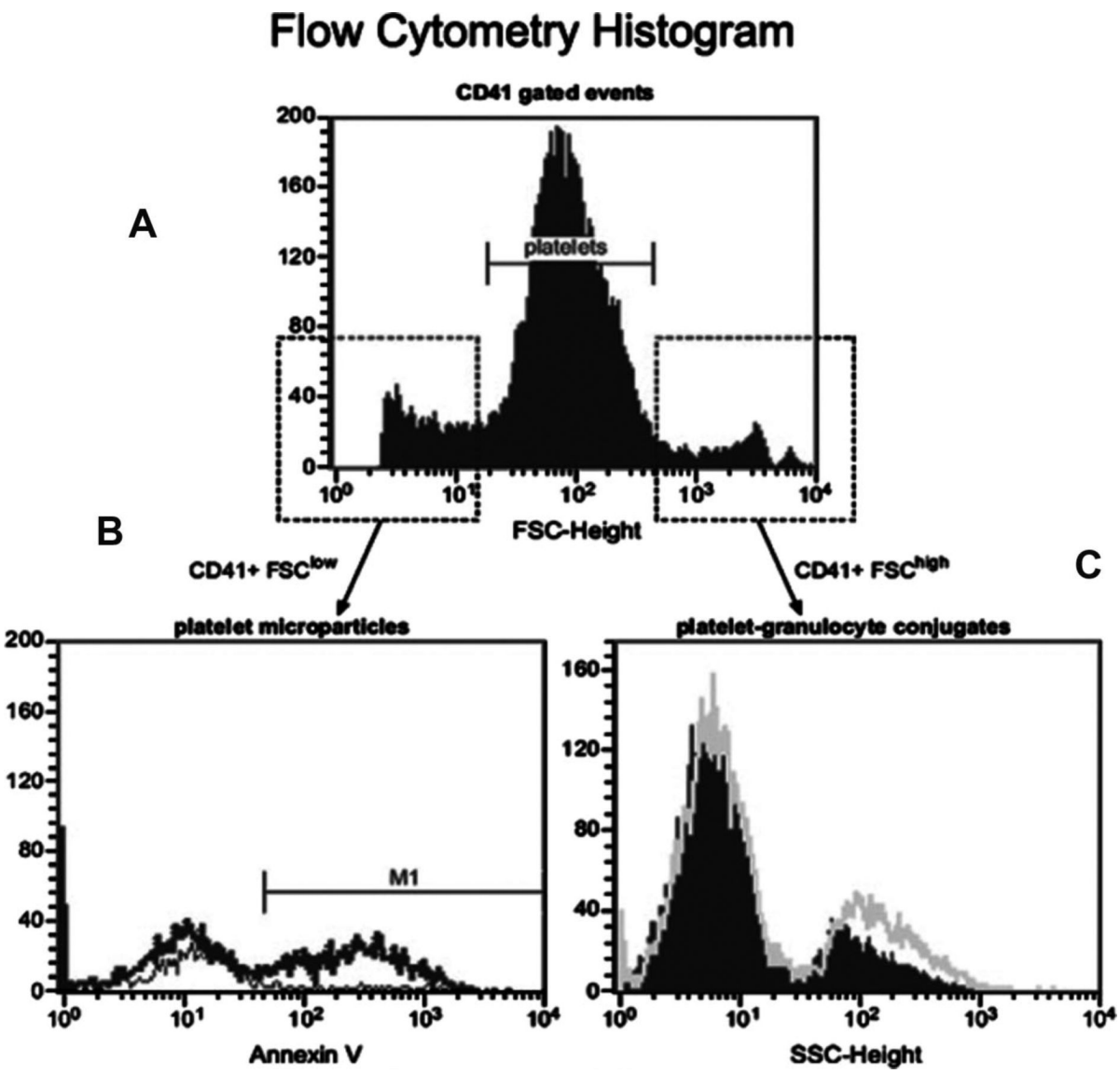

Figure 1.

Histograms from flow cytometric analyses of coronary sinus blood obtained from a representative patient from the aprotinin and placebo group. Three CD41a+ populations were found based on forward scatter characteristics (FSCs): microparticles ($CD41^+$, FSC^{low}), single platelets, and platelet conjugates ($CD41^+$, FSC^{high}) (A). Platelet-derived microparticles were defined as $CD41^+$, annexin V^+ events from the overall microparticle population, and quantified by the M1 gate shown (B). The aprotinin group (thin line) showed significantly less platelet-derived microparticles in coronary sinus blood after off-pump coronary artery bypass (OPCAB) surgery versus the placebo group (thick line). Side-scattering characteristics (SSCs) were used to stratify the platelet conjugate population into a subset with higher granularity (arrow). This $CD41^+$, FSC^{high}, SSC^{high} population, representing platelet-granulocyte conjugates, was significantly reduced by aprotinin (black area) versus placebo (gray area).

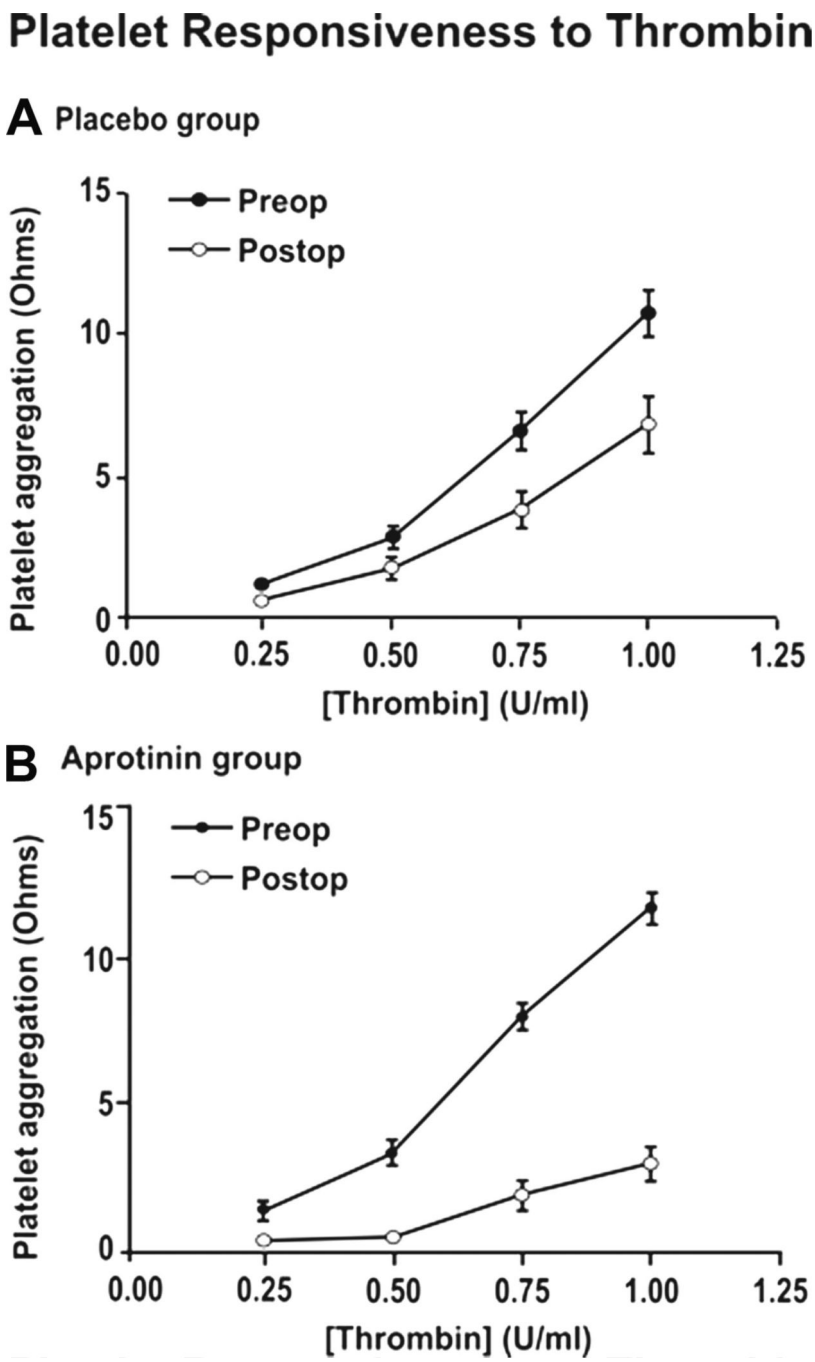

Figure 2.

Ex vivo analysis of blood samples by whole blood aggregometry was used to define platelet responsiveness to thrombin at doses ranging from 0.25 to 1.0 U/mL in both groups before and immediately after off-pump coronary artery bypass (OPCAB) surgery. A slight reduction in the thrombin dose-response relationship was seen in the placebo group after OPCAB surgery compared with baseline (A). In contrast, the aprotinin group showed a significantly greater change in postoperative platelet responsiveness that was specific to thrombin and not seen with other agonists, collagen, and adenosine diphosphate (B).

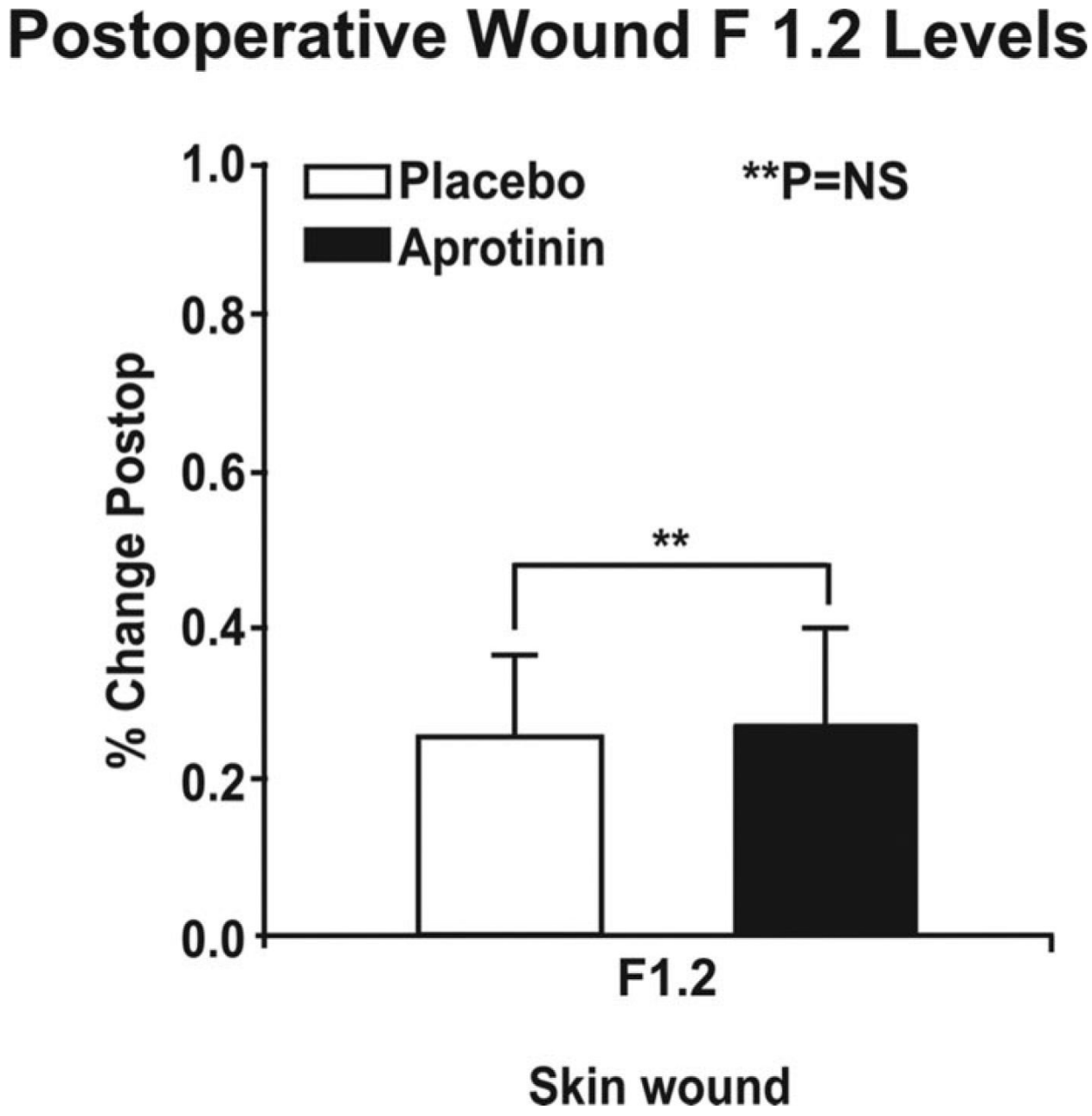

Figure 3.

Blood emanating from a standardized skin wound was collected and analyzed for F1.2 to define the physiological role of thrombin formation in response to microvascular injury. Patients from both the aprotinin and placebo groups showed lower F1.2 levels in these blood droplets after surgery (30 min after protamine administration) compared with baseline. However, this decrease occurred in both groups with no difference noted in the postoperative wound F1.2 levels.

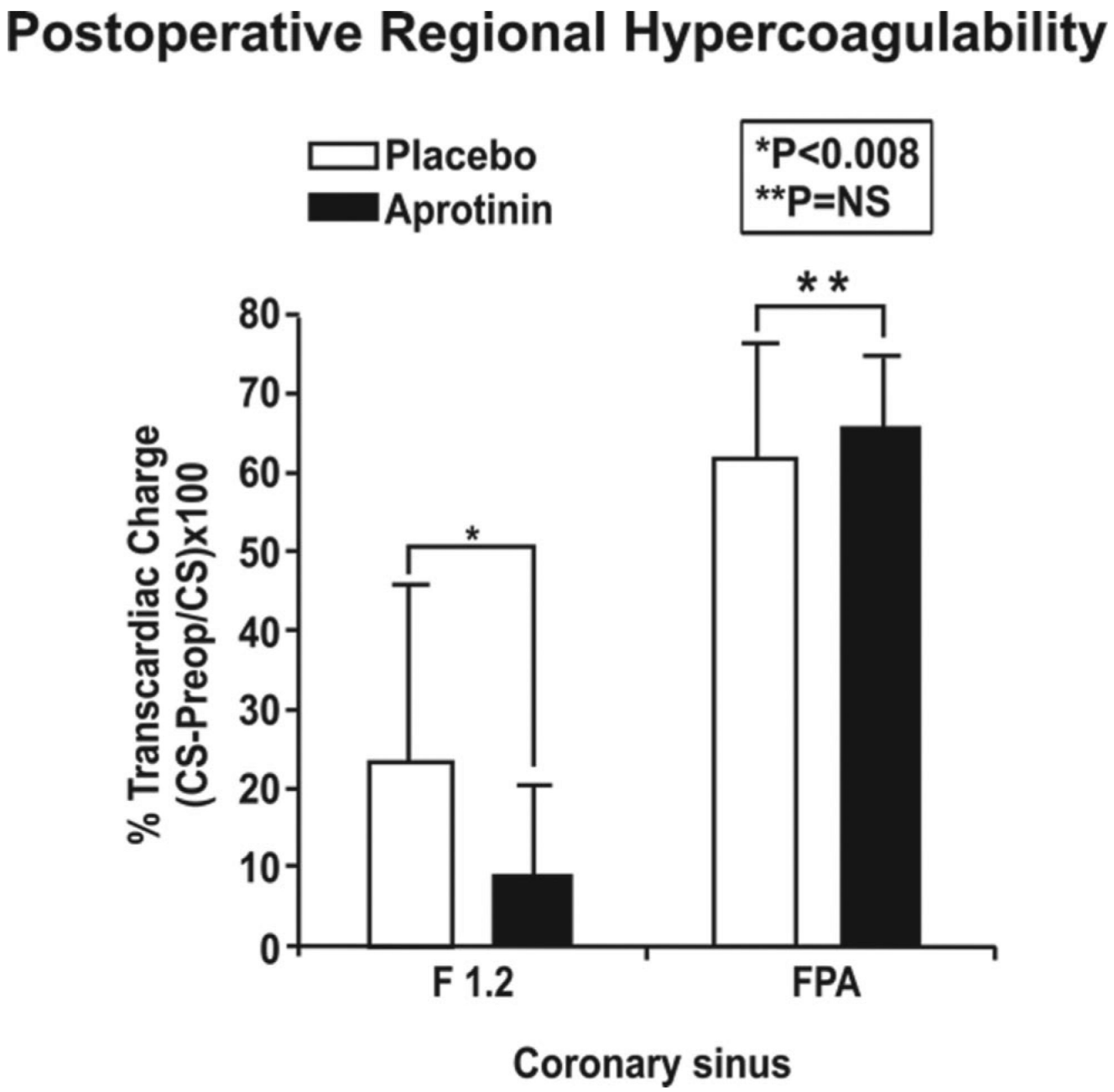

Figure 4.

Blood was simultaneously obtained from the coronary sinus and aorta after the administration of protamine and analyzed for prothrombin fragment F1.2 and fibrinopeptide A (FPA), markers of the formation, and enzymatic activity of thrombin, respectively. The percent transcardiac change in these markers was calculated as a means of defining regional hypercoagulability, an assay previously shown by our group to predict the risk for saphenous vein graft thrombosis. The postoperative gradient for F1.2 was significantly reduced in the aprotinin versus placebo group but no difference was noted for FPA.

Table 1

Bleeding and Red Blood Cell (RBC) Transfusion

	Placebo	Aprotinin	*P*
Intraoperative bleeding	1034 ± 659 mL	794 ± 465 mL	<0.03
First postoperative hour	163 ± 157 mL	91 ± 64 mL	<0.03
First 24 h	810 ± 415 mL	603 ± 330 mL	<0.004
RBC transfusion requirement	0.66 ± 0.78 units	0.39 ± 0.64 units	<0.04

Values are in mean ± SD.

Table 2

Coagulation and Platelet Function

	Placebo	Aprotinin	*P*
TEG®: MA (tissue factor 20 μM)	58 ± 5 mm	60 ± 6 mm	NS
WBA: thrombin 1 U/mL	6.8 ± 5.6 ohms	2.9 ± 2.6 ohms	0.007
WBA: collagen 1 μg/mL	5.15 ± 2.5 ohms	5.29 ± 3.1ohms	NS
WBA: collagen 5 μg/mL	10.99 ± 3.4 ohms	10.12 ± 3.9 ohms	NS
WBA: ADP 5 mM	3.41 ± 1.3 ohms	4.17 ± 2.1 ohms	NS
WBA: ADP 10 mM	6.08 ± 2.1 ohms	6.11 ± 3.2 ohms	NS
F1.2 level in blood[a]	0.26 ± 0.11 μg/mL	0.27 ± 0.13 μg/mL	NS

Values are in mean ± SD.

TEG® = thromboelatography; MA = maximum amplitude; ADP = adenosine diphosphate; WBA = whole blood agrometry.

[a] F1.2 level in blood represents thrombin generation after microvascular injury from a standardized incisional skin wound.

Table 3

Transcardiac Gradients[a]

	Placebo	Aprotinin	*P*
F1.2 levels[b]	23.5% ± 22.3%	9.03% ± 11.2%	<0.008
Fibrinopeptide A	62% ± 14.4%	65.5% ± 9.5	NS
Platelet derived microparticles	15% ± 8%	9% ± 6%	<0.05
Platelet-leukocyte conjugates	33% ± 18%	19% ± 11%	<0.02

Values are in mean ± SD.

[a] The transmyocardial gradient in these markers was analyzed from simultaneously procured blood samples from the aorta (Ao) and coronary sinus (CS) using the equation: ([CS – Ao]/CS) × 100.

[b] F1.2 levels measured 30 min after protamine administration in both groups.