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Perioperative management of aspirin resistance after off-pump coronary artery bypass grafting: possible role for aprotinin

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Abstract

BACKGROUND—Aspirin is the only drug proven to reduce saphenous vein graft (SVG) failure, but aspirin resistance (ASA-R) frequently occurs after off-pump coronary artery bypass grafting (OPCAB). The factors, mechanism, and best means for preventing and/or treating ASA-R have not been established. This study hypothesizes that thrombin production during OPCAB stimulates this acquired ASA-R.

STUDY DESIGN AND METHODS—A nonrandomized prospective cohort of 255 patients (n = 465 SVG) who underwent OPCAB with varied use of aprotinin (21%) and different SVG preparation techniques (standard, 56% vs. low-pressure, 44%) was analyzed. A surplus SVG segment was obtained to assess endothelial integrity. ASA-R was determined at baseline, after surgery, and on Days 1 and 3 by three assays. The effects of aprotinin on thrombin responsiveness were analyzed by means of whole-blood aggregometry, SVG tissue factor (TF) activity, and transcardiac thrombin production (i.e., F1.2 levels in aorta versus coronary sinus). SVG patency was assessed on Day 5 with multichannel CT angiography.

RESULTS—ASA-R developed in 42 percent of patients after OPCAB. Multivariate analysis showed that ASA-R, endothelial integrity, and target size independently predicted early SVG failure. Aprotinin use was associated with: 1) reduced postoperative ASA-R (15%); 2) decreased platelet (PLT) response to thrombin; 3) reduced TF activity within SVG segments; 4) decreased transcardiac thrombin gradient; and 5) improved SVG patency.

CONCLUSION—ASA-R is a common post-OPCAB event whose frequency may be reduced by intraoperative use of aprotinin, possibly via TF and thrombin suppression. Improved perioperative PLT function after OPCAB may also inadvertently enhance the clinical relevance of these potential antithrombotic effects.

Aspirin is the only drug proven to reduce saphenous vein graft (SVG) failure, but aspirin resistance (ASA-R) frequently occurs after off-pump coronary artery bypass grafting (OPCAB). The factors, mechanism, and best means for preventing and/or treating this acquired ASA-R have not been established. Thrombin production occurs during cardiac surgery despite heparin administration, with peak levels seen just after protamine administration.[1,2] Although thrombin production is important for normal hemostasis, its excessive formation plays a pivotal role in the development of consumptive coagulopathy[3] and thrombosis.[4] Thrombin also

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induces a complete platelet (PLT) response during aggregometry despite aspirin use, suggesting its possible involvement in the mechanism of postoperative ASA-R. In vitro or animal models, however, are insufficient for understanding the complex interactions involved in the development of clinical ASA-R. The only study design capable of establishing the mechanistic link of ASA-R to early SVG failure is an adequately powered prospective clinical trial in humans. To date, though, no such study has been conducted.

In a recent study, aprotinin administration during OPCAB was associated with reduced postoperative ASA-R compared with placebo.[5] This study, however, was under-powered to show the effect of aprotinin on SVG failure or to address the mechanism of this intriguing effect on aspirin responsiveness. The prothrombin fragment F1.2 is an established marker of thrombin generation during cardiac surgery.[2] In a previous report from an OPCAB cohort not given aprotinin, patients who developed SVG failure by Day 5 showed a sixfold increase in coronary sinus F1.2 levels just after protamine administration.[6] Although the antifibrinolytic effects of aprotinin are emphasized, new evidence shows it can also modulate the thrombin burst after protamine use, in part via blockade of the PLT thrombin receptor PAR-1 (protease-activated receptor-1).[7,8] The purpose of this interim analysis of our ongoing prospective cohort study was to compare patients who received aprotinin with those who did not receive antifibrinolytic therapy during OPCAB. We hypothesized that diminished perioperative thrombin formation in the aprotinin group is responsible for limiting the development of postoperative ASA-R and SVG failure.

MATERIALS AND METHODS

Patients and procedures

Following institutional review board (IRB) approval in June 2004, all subjects provided informed consent before enrollment into a prospective study investigating early graft failure after OPCAB. From June 2004 until March 2005, 460 patients were screened and 153 patients excluded from the study for the following reasons: creatinine level of more than 2.0 mg per dL (n = 63); requirement of cardiopulmonary bypass (n = 37); refusal of consent (n = 32); and inability to obtain valid consent owing to patient condition or emergent surgery (n = 21). After enrollment, CT angiograms were not obtained in 52 patients for the following reasons: 1) heart rate of more than 100 bpm or a creatinine level of more than 2.0 mg per dL (n = 28); 2) patient withdrawal of consent (n = 12); 3) patient lost to follow-up (n = 10); and 4) patient death without an autopsy to confirm bypass graft patency (n = 2). Based on the preference of the attending surgeon (i.e., nonrandomized assignment), 54 of the 255 patients (20%) who completed the protocol received a modified full-dose regimen of aprotinin during OPCAB: 10,000 kallikrein-inhibiting units (KIU) intravenous test dose (or saline placebo), 2 million KIU aprotinin before sternotomy, and 500,000 KIU per hour until the end of the operation. A comparison with subjects who did not receive aprotinin during OPCAB (n = 201) serves as the basis of this report.

Demographics, medications, preoperative risk factors, intraoperative, and postoperative data were prospectively recorded onto case report forms (Teleform, TELEform Elite, Cardiff Software Ltd, Vista, CA), electronically scanned, and imported into a relational database.

Surgical technique

Four surgeons, all experienced in OPCAB, were responsible for patient enrollment. After median sternotomy, the left internal thoracic artery was used in all the patients. The saphenous vein was harvested using an endoscopic (n = 420 venous conduits; VasoView5, Guidant Systems, Inc., Minneapolis, MN) or open (n = 45 venous conduits) approach, based on anatomic considerations. To prepare for grafting, procured SVGs were initially flushed with

heparinized saline by use of syringe injection with no method employed to control the distending pressure (54%). Based on the importance of endothelial integrity,[9] the remainder of the SVGs in the study were flushed with saline at a distending pressure maintained at less than 100 mmHg (46%). The proximal aortosaphenous anastomoses were performed first with a partial occluding aortic clamp to minimize the variations in SVG ischemic times. The distal anastomoses were then performed using suction-based exposure and stabilizing devices (Octopus 4.3, Medtronic Inc., Minneapolis, MN). Heparin was given initially and every 30 minutes at a dose calculated to obtain an activated clotting time of more than 300 seconds and a heparin level of more than 2 IU per mL according to protamine titration (HMS heparin assay cartridges, Medtronic Inc.). Heparin was reversed by half the dose of protamine calculated by heparin-protamine titration. Preoperative aspirin (325 mg orally/day) was continued and given within 6 hours of surgery as the sole antithrombotic agent.

Intraoperative blood flow analysis

Blood flow and flow waveform were measured in each graft with transit-time ultrasound (Medi-Stim USA, Inc., Brooklyn Park, MN). Waveforms were analyzed for pulsatility index (PI = maximum-minimum/mean blood flow) and percentage diastolic flow with data acquisition software (WinDaq, DATAQ Instruments, Inc., Dayton, OH). Grafts with flow of less than 10 mL per min and a pulsatility index of more than 5 despite anastomotic revision were excluded from analysis (n = 4).

Analysis of conduit phenotype

Surplus segments obtained from each SVG just before the distal anastomosis were stored in Hank's balanced salt solution, embedded in cutting compound (Tissue-Tek O.C.T., Redding, CA), and frozen in liquid nitrogen. In all segments, percent luminal circumference staining for the endothelial marker CD31 (R&D System, Inc., Minneapolis, MN) and tissue factor (TF; United States Biological, Swampscott, MA) were analyzed as described previously.[9,10] Additional SVG sections (n = 62) were immediately analyzed for TF activity.[11] Undistended segments of vein (n = 5), procured before surgical preparation, were analyzed as a negative control. The assay was initiated by isolation of the endothelial surface in a custom assay chamber for exposure to 50 mmol per L Tris-HCl, 2 U per mL Factor (F)VII (American Diagnostica, Greenwich, CT), 2 U per mL FX (American Diagnostica), and 50 mmol per L calcium chloride (Sigma Chemical Co., St Louis, MO). Secure fixation of the vein to the chamber prevented exposure of the adventitial surface to the reaction mixture. After a 20-minute incubation period, the reaction was stopped by the addition of ethylenediaminetetraacetate (Sigma). The amount of activated FX (FXa) in the supernatant was then measured by the addition of a commercial chromogenic substrate (Spectrozyme FXa, American Diagnostica), for 3 minutes. After the addition of glacial acetic acid (Sigma), the absorption was measured in a spectrophotometer (Becton-Dickinson, Franklin Lakes, NJ) at 405 nm, and the amount of FXa generated in the assay chamber was determined from a standard curve. Each assay was performed in duplicate in the absence and presence of 500 KIU per mL aprotinin.

Assays for coagulation

Tests of coagulation (international normalized ratio, partial thromboplastin time, fibrinogen and F1.2 levels, and quantitative D-dimer levels) were obtained from citrated blood samples drawn just before skin incision (baseline), just after protamine administration (postoperation), and on Postoperative Days 1, 3, and 30. In addition, a coronary sinus blood sample drawn approximately 30 minutes after bypass grafting in a subset of subjects (n = 40) by direct puncture with a 21-gauge needle was also analyzed for F1.2 with an enzyme-linked immunosorbent assay (ELISA) kit (Enzygnost F1.2 micro, Dade-Behring, Deerfield, IL). As

previously described,[12] comparison of F1.2 in the coronary sinus ($F1.2_{CS}$) with a simultaneously obtained aortic sample ($F1.2_{Ao}$) allows for calculation of percent transcardiac change as follows:

$$(F1.2_{CS} - F1.2_{Ao})/F1.2_{CS} \times 100.$$

To control for factors that could influence intramyocardial thrombin formation other than a diseased bypass conduit, percent transcardiac change in F1.2 was also assessed from CABG patients preoperatively before heparin (n = 4) and after heart transplantation after protamine administration (n = 8; institutional review board number H-22721).

Assays for PLT function

ASA-R was established based on positive findings consistent with this diagnosis on at least two of three of the following assays: 1) PLT mapping studies by thromboelastography (Haemoscope, Niles, IL); 2) PLT response in whole-blood aggregometry (Chronolog, Havertown, PA) to 1 μg per mL versus 5 μg per mL collagen; and 3) 11-dehydro-TXB2 levels in PLT-poor serum with an ELISA kit (Assay Designs Inc., Ann Arbor, MI). Exact diagnostic criteria for each of these assays have been reported previously.[5]

Additionally, the effect of aprotinin on PLT response was assayed by the addition of thrombin (0.25 or 0.5 U/mL) and GPRP (glycl-prolyl-arginyl-proline), followed by collagen (5 μg/mL) after 6 minutes, in the presence or absence of aprotinin (300 KIU/mL).

Postoperative graft follow-up

SVG patency was determined by two thoracic radiologists (CW, JJ) in a blinded, independent review of an angiographic study acquired prior to hospital discharge, with a 16-slice multidetector CT scan (MX8000IDT, Philips Medical, Andover, MA). The protocol for data acquisition and analysis was performed as previously reported by our group.[13]

Statistical analysis

The primary endpoint of this study was the risk of early graft failure in patients treated intraoperatively with aprotinin compared with patients who did not receive the drug. In a recently reported randomized trial of aprotinin versus placebo, the graft failure rates at midterm follow-up were 3 and 17 percent, respectively.[14] Assuming that aprotinin produces a minimum twofold difference in graft patency in this cohort, power analysis indicated that enrollment of 105 patients per group was needed for 80 percent power and two-sided p = 0.05 (http://calculators.stat.ucla.edu/powercalc/).

All risk variables were subjected to univariate and multivariate logistic regression analyses. Numerical variables were compared by means of the unpaired t test, and the Fisher's exact test was used to compare categorical variables. Variables with a p value of less than 0.1 at univariate analysis were then used as independent variables in a stepwise logistic regression analysis, with a p value of 0.05 the criterion for retention of variables in the final model. Statistical analysis was performed with a statistical package (In-Stat, Scottsdale, AZ) with the assistance of a biostatistician (LN). The study sponsors had no role in the publication of these data.

RESULTS

Patients

CT angiographic follow-up was completed in 255 of 306 (82%) enrolled subjects. Early patency of arterial grafts (n = 261; left internal mammary artery, 223; right internal mammary

artery, 22; radial, 16) is not included in the analysis, but was 100 percent. Of 469 evaluable SVGs, 4 with intraoperative graft blood flow of less than 10 mL per minute were excluded from the analysis despite revision. Of the 465 analyzed SVGs, 16 grafts in 16 patients were found to be thrombosed. These included 6 of 195 grafts placed to targets on the right coronary artery, 6 of 164 grafts to the circumflex distribution, and 4 of 106 placed onto a diagonal coronary artery. In 4 patients, graft occlusion was also confirmed by conventional catheter-based angiography.

Aprotinin use during OPCAB was limited to 54 of 98 cases (55%) enrolled by RP. Other than operative surgeon, the aprotinin group showed no significant differences in demographics, preoperative risk factors or medications given before or after surgery compared with the control group, that is, group not administered aprotinin or any other antifibrinolytic agent. Intraoperative data such as conduit flow and endothelial integrity, vein diameter, target size and quality, need for intraaortic balloon pumping, or inotropic requirements were also similar. Moreover, the total number of grafts in both aprotinin recipients and nonrecipients was the same (3.5 ± 0.3 vs. 3.2 ± 0.7; p = NS).

Changes in PLT reactivity

In vivo treatment with aprotinin—Postoperatively, both PLT count and function (defined by maximum amplitude and change in impedance by thromboelastography and whole-blood aggregometry) dropped relative to baseline values in 92 percent of all subjects and returned to baseline in 78 percent by Day 3. No detectable differences were noted between treatment groups (Table 1). Although the incidence of ASA-R was negligible at baseline and on Postoperative Day 30 (≤3% for both groups at both time points), aprotinin-treated patients were significantly less likely to acquire postoperative ASA-R by Postoperative Day 3 (aprotinin, 15% vs. placebo, 42%; p = 0.001).

In vitro—Compared with untreated blood samples, aprotinin treatment blocked the aggregation response to low- and high-dose thrombin (change in ohms, 3.4 ± 4.3 vs. 14.5 ± 5.2 and 4.6 ± 3.5 vs. 18.4 ± 5.9 for aprotinin vs. control groups at low and high thrombin doses, respectively; n = 5/group, p < 0.01 for both). In contrast, the ensuing aggregation response to collagen after thrombin challenge was better preserved in the aprotinin group than in the control group (change in ohms, 15.4 ± 5.9 vs. 4.3 ± 1.3, respectively, p < 0.05; n = 5/group; Fig. 1).

Changes in TF activity

Negligible TF activity was found in the undistended control veins (1.4 ± 0.8 U/cm^2, n = 5). SVGs that were prepared for grafting with the standard techniques (i.e., uncontrolled pressure via syringe injection by hand) showed luminal TF activity that was significantly higher than that seen in veins prepared with a pressure-controlled technique (4.6 ± 2.4, n = 25 vs. 12.1 ± 3.8 U/cm^2, n = 46; p < 0.05). The addition of aprotinin was found to block TF activity induced by pressure injury (6.6 ± 1.3, n = 20; p < 0.05 vs. no aprotinin; Fig. 2).

Changes in coagulation

Despite equivalent intraoperative levels of heparin (2.3 ± 0.5 vs. 2.4 ± 0.9 U/mL; p = NS) and activated clotting time (data not shown), aprotinin administration significantly reduced the percent perioperative change in thrombin formation (60 ± 44% vs. 116 ± 91% change in F1.2; p = 0.04). The percent transmyocardial difference in F1.2 after bypass grafting was also significantly reduced in patients given aprotinin therapy (24 ± 37%, n = 10 vs. 82 ± 42%, n = 13; p = 0.04; Fig. 3). The effect of aprotinin on this gradient was greatest in patients with the combination of low graft blood flow and poor SVG endothelial integrity (Fig. 4).

Graft patency

Abnormal flow parameters (flow <10 mL/min and pulsatility index >5 or % diastolic flow <50) were detected in 12 grafts before revision and four grafts after revision. After the exclusion of these four grafts, multivariate analysis found that the development of ASA-R, target size, and endothelial integrity were significant predictors of early graft failure with odd ratios of 2.41 (1.10–3.89), 0.65 (0.23–0.91), and 5.22 (95% CI [confidence interval], 1.72–8.90), respectively. Graft failure was observed in 14 patients in the control group (6% of grafts) and 2 patients in the aprotinin group (2% of grafts), representing a significant difference ($p = 0.037$, Fisher's exact test). Aprotinin use during OPCAB was not an independent predictor of SVG failure, likely because of its inverse association with ASA-R, thereby limiting the additional predictive information it provides given the model used.

DISCUSSION

The main finding of this prospective cohort study was a significant reduction in postoperative ASA-R and early SVG failure after OPCAB associated with intraoperative aprotinin use. In a prior analysis that excluded aprotinin-treated patients, we found that the combination of ASA-R and SVGs with endothelial disruption led to a synergistic increase in the risk of early failure after OPCAB.[6,9] This more recent multivariate analysis shows a reduction in SVG failure in aprotinin-treated patients, an antithrombotic effect that appears to be dependent on aprotinin's ability to abrogate postoperative ASA-R. We propose that a greater transcardiac gradient of F1.2 in the absence of aprotinin signifies local thrombin deposition within microclots formed in the SVG soon after perfusion. It has been shown that clot-bound thrombin becomes resistant to inhibition by heparin, suggesting that current standard therapy (aspirin and heparin without aprotinin) is suboptimal against thrombosis.

A clear understanding of the relationship between aprotinin use and SVG patency requires an understanding of the predominant mechanism of early graft failure in OPCAB. Technical defects in the anastomosis are often blamed, particularly after OPCAB. When intraoperative flow readings are used to revise and/or exclude defective grafts from analysis, however, we have found that the regulation of thrombin formation within the SVG may play a relatively more important role.[6] Abrupt withdrawal of heparin therapy, required to achieve hemostasis, has long been described to be associated with a burst in thrombin formation.[15] Several studies have shown that aprotinin effectively reduces thrombin formation after protamine dosing.[16,17] In this study, we demonstrated an effect on transcardiac thrombin formation, producing an aprotinin benefit localized to within the SVG. The ability of aprotinin to preserve PLT sensitivity to aspirin in this trial corroborates findings from our smaller, randomized clinical trial.[5] Taken together, these data provide a solid rationale for ongoing studies to establish the link between our increasing appreciation of the antithrombotic actions of aprotinin and improved SVG patency after OPCAB.

Because aprotinin and OPCAB are associated with improved hemostasis, both have been implicated as causes of early SVG failure.[18] This fear is based on the largely unsubstantiated belief that postoperative bleeding and thrombosis are mutually exclusive, that is, a lower risk of bleeding leads to a higher risk of thrombosis. The absence of an accepted assay for the hypercoagulable state prevents critical evaluation of this premise in vivo. A growing body of evidence, however, supports the idea that aprotinin has both antithrombotic and hemostatic effects. Although aprotinin is traditionally thought to limit thrombin production solely through the inhibition of the intrinsic coagulation cascade,[19] our analyses corroborate the work of other investigators demonstrating that it also directly inhibits the extrinsic cascade.[20,21] Moreover, the specific suppression of the PLT aggregation response to thrombin illustrates that aprotinin blocks the thrombin receptor on the PLT, namely, PAR-1.[7,8] As a result, aprotinin has been shown to reduce in vivo arterial thrombosis in a recently reported animal model.[20]

Clinical thrombosis is increased in patients with excessive thrombin formation[15] or ASA-R,[22] and their combination in vitro has been shown to be mutually stimulatory.[23] If aprotinin inhibits both processes (as we suggest), then why have multiple randomized trials performed to date failed to show the benefits of aprotinin with respect to thrombotic endpoints such as acute SVG loss[24] or perioperative myocardial infarct?[25] It is possible that the absence of cardiopulmonary bypass changes the equation by reducing postoperative PLT dysfunction after OPCAB.[26] Relatively intact PLTs may inadvertently aggravate the potential for synergy with thrombin production within the lumen of the SVG, thus increasing the risk of graft failure believed to be associated with OPCAB[18,26] and providing a unique population to test the antithrombotic effects of aprotinin.

Thrombin formation in cardiac surgery patients has been attributed to surgical trauma,[2] contact activation of blood from the cardiopulmonary bypass circuit,[1] ischemia-reperfusion injury,[12] and unstable coronary artery disease.[6] Despite prior studies demonstrating that pressure damage to the SVG causes the luminal expression of TF,[11] the primary stimulus for thrombin, the contribution of the newly grafted SVG to perioperative thrombin formation, has been largely ignored. By demonstrating a direct relationship to graft flow and SVG endothelial integrity, our data suggest that the transcardiac gradient of F1.2 after protamine reversal directly relates to those factors classically referred to as “Virchow’s triad.” We found a very low transcardiac gradient of F1.2 in heart transplant and preoperative CABG patients, suggesting that ischemia-reperfusion injury and coronary artery disease play a minor role. As opposed to isolated measurements of thrombin formation, the simultaneous comparison of F1.2 in the aortic and coronary sinus (i.e., transcardiac gradient) accounts for factors that affect marker clearance, hemodilution, blood loss, and the effect of retransfused shed mediastinal blood. The reduced F1.2 gradient seen in aprotinin recipients suggests that this agent might interrupt the dysregulated feedback between ASA-resistant PLTs and thrombin deposition in SVGs expressing high levels of TF.

This study has several limitations. First, the patients were not randomly assigned to their treatment groups, resulting in only one surgeon enrolling patients into the aprotinin group. The protocol, however, included routine transit-time flow measurements, revision of grafts with poor readings, and exclusion of grafts without adequate improvement. As a result, multivariate analysis detected no surgeon influence on the patency results. Second, the clinical relevance of ASA-R and best method of diagnosis remain uncertain. We addressed this problem by considering the diagnosis of ASA-R established only if findings were compatible in two of three separate assays performed in all patients. In previous studies, we demonstrated a strong correlation of these assays with flow cytometry, a more established method for quantifying PLT activation, thus justifying confidence in our diagnostic criteria. Third, analyzing graft patency just before hospital discharge did not capture all thrombotic events in this cohort. Graft patency follow-up studies performed in a subset of patients who underwent repeat CT angiography at 6 to 12 months revealed five additional graft failures. These failures, however, represented only 2 percent of the grafts that were evaluated, confirming that the greatest risk period remains the first postoperative week.[27] Our protocol of predischarge CT angiography provided a higher and more uniform follow-up—an acceptable tradeoff, in our estimation. Finally, a wide range of aprotinin-related anti-inflammatory actions that may also influence the postoperative coagulant response were not assessed during this study. Future randomized studies appropriately powered to assess the limited effect of aprotinin on graft patency witnessed in this study are being planned to investigate this important potential mechanism of effect. On the basis of these limitations, our findings, while mechanistically consistent, should be considered only hypothesis-generating pending prospective confirmation.

In conclusion, aprotinin administration in this prospective cohort study was associated with a reduced incidence of new ASA-R and local thrombin formation, as well as less early SVG

failure after OPCAB. These data corroborate the findings of a recently reported randomized trial of aprotinin use during OPCAB[5] and add to the growing body of evidence that aprotinin demonstrates both hemostatic and antithrombotic effects.[28] If these results are confirmed in future studies, aprotinin may prove to be a valuable therapeutic adjunct to promote conduit patency after OPCAB.

ABBREVIATIONS

ASA-R
: aspirin resistance

FXa
: activated Factor X

KIU
: kallikrein-inhibiting units

OPCAB
: off-pump coronary artery bypass grafting

SVG(s)
: saphenous vein graft(s)

References

1. Dixon B, Santamaria J, Campbell D. Coagulation activation and organ dysfunction following cardiac surgery. Chest 2005;128:229–36. [PubMed: 16002940]
2. Boisclair MD, Lane DA, Philippou H, Esnouf MP, Sheikh S, Hunt B, Smith KJ. Mechanisms of thrombin generation during surgery and cardiopulmonary bypass. Blood 1993;82:3350–7. [PubMed: 8241505]
3. Despotis GJ, Avidan MS, Hogue CW Jr. Mechanisms and attenuation of hemostatic activation during extracorporeal circulation. Ann Thorac Surg 2001;72:S1821–31. [PubMed: 11722116]
4. Pothula A, Serebruany VL, Gurbel PA, McKenzie ME, Atar D. Pathophysiology and therapeutic modification of thrombin generation in patients with coronary artery disease. Eur J Pharmacol 2000;402:1–10. [PubMed: 10940351]
5. Poston RS, Gu J, White C, Brown J, Gammie J, Pierson RN, Lee A, Connerney I, Avari T, Christenson R, Tandry U, Griffith BP. Aprotinin shows both hemostatic and anti-thrombotic effects during off-pump coronary artery bypass grafting. Ann Thorac Surg 2006;81:104–10. [PubMed: 16368345]
6. Poston RS, White C, Gu J, Read K, Gammie J, Brown J, Lee A, Griffith B, Pierson R III. Endothelial injury and acquired aspirin resistance as promoters of regional thrombin formation and early vein graft failure after coronary artery bypass grafting. J Thorac Cardiovasc Surg 2006;131:22–30.
7. Day JR, Punjabi PP, Randi AM, Haskard DO, Landis RC, Taylor KM. Clinical inhibition of the seven-transmembrane thrombin receptor (PAR1) by intravenous aprotinin during cardiothoracic surgery. Circulation 2004;110:2597–600. [PubMed: 15262827]
8. Poullis M, Manning R, Laffan M, Haskard DO, Taylor KM, Landis RC. The antithrombotic effect of aprotinin: actions mediated via the proteaseactivated receptor 1. J Thorac Cardiovasc Surg 2000;120:370–8. [PubMed: 10917956]
9. Manchio JV, Gu J, Romar L, Brown J, Gammie J, Pierson RN, Griffith B, Poston RS. Disruption of graft endothelium correlates with early failure after off-pump coronary artery bypass surgery. Ann Thorac Surg 2005;79:1991–8. [PubMed: 15919298]
10. Poston R, Gu J, Manchio J, Lee A, Brown J, Gammie J, White C, Griffith BP. Platelet function tests predict bleeding and thrombotic events after off-pump coronary bypass grafting. Eur J Cardiothorac Surg 2005;27:584–91. [PubMed: 15784355]

11. Muluk SC, Vorp DA, Severyn DA, Gleixner S, Johnson PC, Webster MW. Enhancement of tissue factor expression by vein segments exposed to coronary arterial hemodynamics. J Vasc Surg 1998;27:521–7. [PubMed: 9546239]
12. Kalweit G, Bach J, Huwer H, Winning J, Hellstern P. The impact of cardiac ischemia and reperfusion on markers of activated haemostasis and fibrinolysis during cardiopulmonary bypass: comparison of plasma levels in arterial and coronary venous blood. Thromb Res 2005;116:33–9. [PubMed: 15850606]
13. Frazier AA, Qureshi F, Read KM, Gilkeson RG, Poston RS, White CS. Coronary artery bypass grafts: assessment with multidetector CT in the early and late postoperative setting. Radiographics 2005;25:881–96. [PubMed: 16009813]
14. Poston RS, White C, Gu J, Brown J, Gammie J, Pierson RN, Lee A, Connerney I, Avari T, Christenson R, Tandry U, Griffith BP. Safety and efficacy of aprotinin in off-pump coronary artery bypass grafting (OPCAB): a double-blind, placebo-controlled randomized trial. Circulation 2004;110:III-546.
15. Granger CB, Miller JM, Bovill EG, Gruber A, Tracy RP, Berrios E, Green C, Krucoff M, Harrington RA, Ohman EM, Califf RM. Rebound increase in thrombin generation and activity after cessation of intravenous heparin in patients with acute coronary syndromes. Circulation 1995;91:1929–35. [PubMed: 7895349]
16. Dietrich W. Reducing thrombin formation during cardiopulmonary bypass: is there a benefit of the additional anticoagulant action of aprotinin? J Cardiovasc Pharmacol 1996;27(Suppl 1):S50–7. [PubMed: 8938284]
17. Dietrich W, Dilthey G, Spannagl M, Jochum M, Braun SL, Richter JA. Influence of high-dose aprotinin on anticoagulation, heparin requirement, and celite- and kaolin-activated clotting time in heparin-pretreated patients undergoing open-heart surgery. A double-blind, placebo-controlled study. Anesthesiology 1995;83:679–89. [PubMed: 7574046]
18. Mariani MA, Gu Y, Boonstra PW, Grandjean JG, van Oeveren W, Ebels T. Procoagulant activity after off-pump coronary operation: is the current anticoagulation adequate? Ann Thorac Surg 1999;67:1370–5. [PubMed: 10355414]
19. Hoffmann H, Siebeck M, Thetter O, Jochum M, Fritz H. Effect of aprotinin and C1-esterase inhibitor on activation of the plasma kallikreinkinin system in vivo. Prog Clin Biol Res 1987;236A:159–64. [PubMed: 2441405]
20. Khan MM, Gikakis N, Miyamoto S, Rao AK, Cooper SL, Edmunds LH Jr, Colman RW. Aprotinin inhibits thrombin formation and monocyte tissue factor in simulated cardiopulmonary bypass. Ann Thorac Surg 1999;68:473–8. [PubMed: 10475415]
21. Chabbat J, Porte P, Tellier M, Steinbuch M. Aprotinin is a competitive inhibitor of the factor VIIa-tissue factor complex. Thromb Res 1993;71:205–15. [PubMed: 7692618]
22. Gum PA, Kottke-Marchant K, Poggio ED, Gurm H, Welsh PA, Brooks L, Sapp SK, Topol EJ. Profile and prevalence of aspirin resistance in patients with cardiovascular disease. Am J Cardiol 2001;88:230–5. [PubMed: 11472699]
23. Vanschoonbeek K, Feijge MA, Van Kampen RJ, Kenis H, Hemker HC, Giesen PL, Heemskerk JW. Initiating and potentiating role of platelets in tissue factor-induced thrombin generation in the presence of plasma: subject-dependent variation in thrombogram characteristics. J Thromb Haemost 2004;2:476–84. [PubMed: 15009466]
24. Alderman EL, Levy JH, Rich JB, Nili M, Vidne B, Schaff H, Uretzky G, Pettersson G, Thiis JJ, Hantler CB, Chaitman B, Nadel A. Analyses of coronary graft patency after aprotinin use: results from the International Multicenter Aprotinin Graft Patency Experience (IMAGE) trial. J Thorac Cardiovasc Surg 1998;116:716–30. [PubMed: 9806378]
25. Sedrakyan A, Treasure T, Elefteriades JA. Effect of aprotinin on clinical outcomes in coronary artery bypass graft surgery: a systematic review and meta-analysis of randomized clinical trials. J Thorac Cardiovasc Surg 2004;128:442–8. [PubMed: 15354106]
26. Moller CH, Steinbruchel DA. Platelet function after coronary artery bypass grafting: is there a procoagulant activity after off-pump compared with on-pump surgery? Scand Cardiovasc J 2003;37:149–53. [PubMed: 12881156]

27. Poston RS, White C, Read K, Griffith B. Quantifying neointimal hyperplasia in aortocoronary saphenous vein bypass grafts using multichannel CT angiography [abstract]. J Am Coll Surg 2005;201:S25–6.

28. Landis RC, Asimakopoulos G, Poullis M, Haskard DO, Taylor KM. The antithrombotic and antiinflammatory mechanisms of action of aprotinin. Ann Thorac Surg 2001;72:2169–75. [PubMed: 11789829]

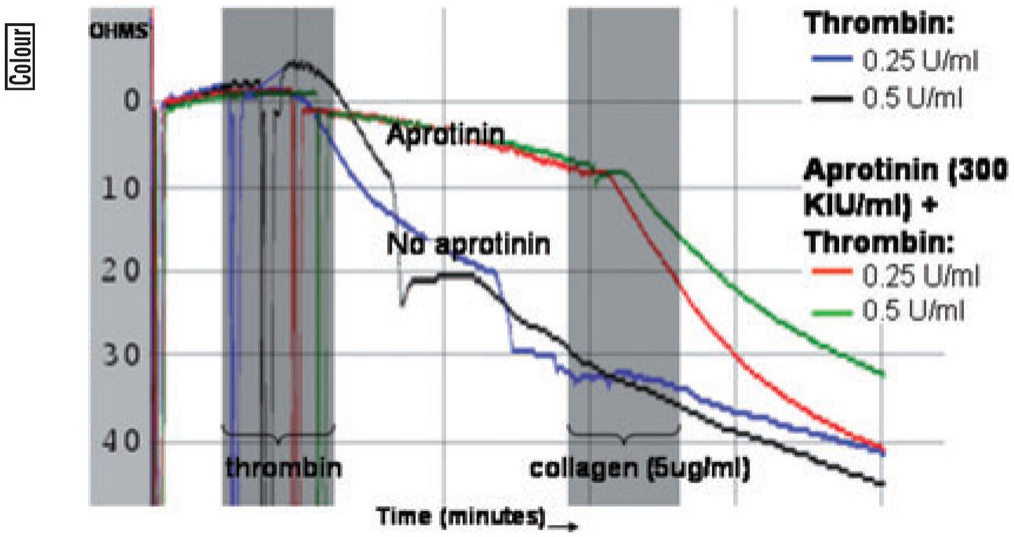

Fig. 1.

Aprotinin inhibits thrombin-induced PLT aggregation and preserves proteolysis-independent pathways of PLT activation. Citrated whole blood was untreated or treated with 200 KIU per mL aprotinin immediately before the addition of thrombin at low (0.25 U/mL) or high doses (0.5 U/mL), followed by the addition of collagen (5 μg/mL) at 6 minutes. After a strong aggregation response to thrombin, illustrated in this representative example by the strong change in impedance (ohms at 6 min), the untreated blood showed a much weaker subsequent response to collagen. In contrast, the aprotinin treatment blocked the response to thrombin while preserving the ensuing aggregation response to collagen.

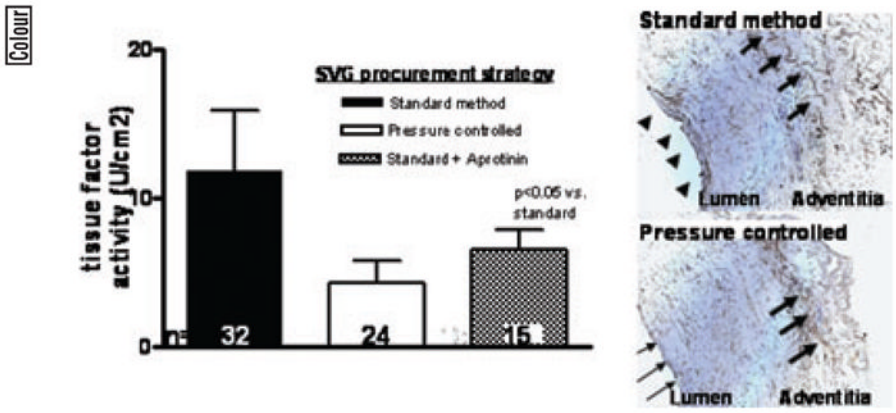

Fig. 2.

Aprotinin blocks the activity of TF expressed on the luminal surface of pressure-injured SVG. SVGs were prepared for grafting with two techniques (pressure-controlled or syringe injection). Surplus SVG segments were analyzed for TF expression with immunohistochemistry and a functional assay. TF was constitutively expressed in the adventitia (thick arrows) but not on the luminal surface of SVG (thin arrows) procured by the pressure-controlled technique. Uncontrolled pressure (syringe injection) resulted in a significant increase in TF expression (arrowheads) and activity, which was blocked by the addition of 500 KIU per mL aprotinin (n = 5/group).

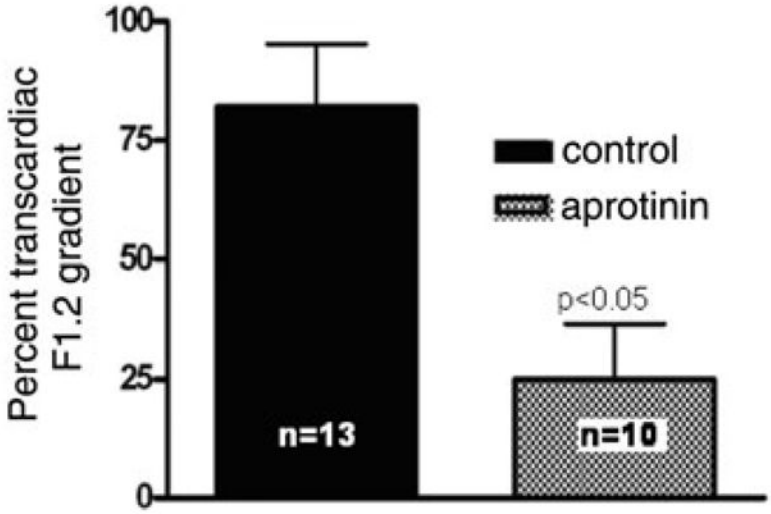

Fig. 3.

Aprotinin administration reduced thrombin formation in the bypass graft. In vivo thrombin generation in the bypass graft was estimated by measuring the gradient of prothrombin fragment F1.2 across the heart (coronary sinus vs. aortic sample) after protamine administration. Patients who received aprotinin showed a significantly lower transcardiac gradient of thrombin ($p < 0.05$).

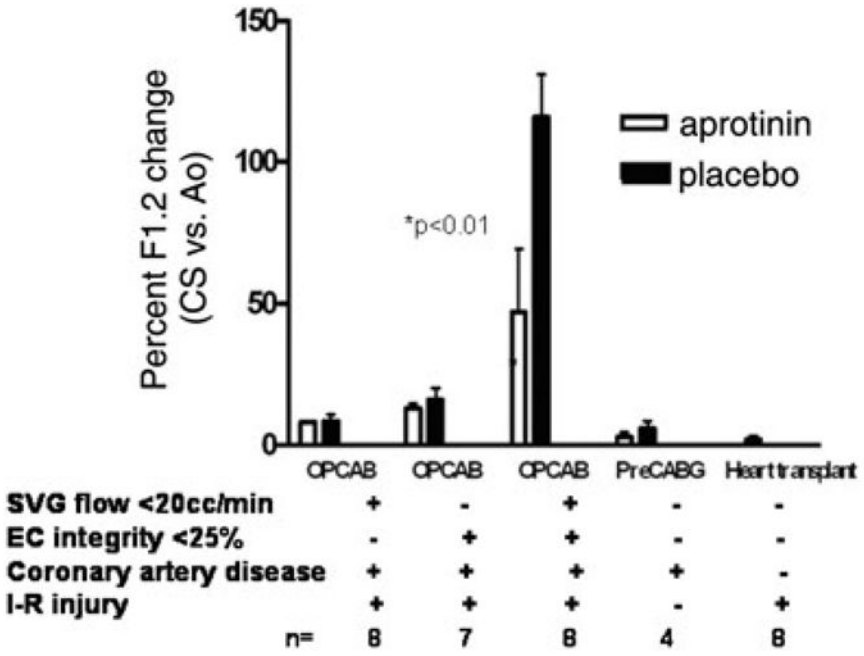

Fig. 4.

Aprotinin limits the burst in thrombin formation that occurs in SVG at high risk for thrombosis. The transcardiac gradients of thrombin formation, defined by the percentage difference in coronary sinus (CS) versus aortic (Ao) levels of F1.2, were measured in OPCAB patients after protamine administration. Only the subgroup of patients who had a graft with low intraoperative flow (<20 mL/min) and poor endothelial integrity (<25% luminal coverage with CD31) showed a significant gradient of thrombin formation and this gradient was reduced by aprotinin use. In contrast, other OPCAB groups developed very little postoperative gradient of F1.2. There was also little transcardiac gradient noted in preoperative OPCAB and postoperative heart transplant patients who served as controls for the influence of coronary artery disease and reperfusion injury, respectively.

TABLE 1

Conduit and coagulation parameters in aprotinin versus control group

Assay	Aprotinin group (n = 54)	Control group (n = 201)	p Value
Endothelium			
% CD31 staining*	51.2 ± 16.9	49.5 ± 19.7	NS
Coagulation			
TEG-MA (mm)†	71.1 ± 5.1	70.4 ± 3.7	NS
WBA—5 μg collagen†	11 ± 4	12 ± 3	NS
Fibrinogen (mg/dL)†	693 ± 104	643 ± 132	NS
D-dimer (ng/mL)†	1643 ± 226	1769 ± 197	NS
Perioperative change in F1.2 (%)*	60 ± 44	116 ± 91	0.04
Flow			
Mean flow*	38 ± 31	40 ± 39	NS
Pulsatility index*	2.5 ± 0.6	2.8 ± 0.5	NS
Percent diastolic flow*	54 ± 14	58 ± 18	NS

* Perioperative changes in F1.2, SVG endothelial integrity, and blood flow were assessed intraoperatively.

† Coagulation tests were compared at their peak levels on Postoperative Day 3.

TEG-MA = thromboelastography PLT mapping; WBA = whole-blood aggregometry.