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Serine proteases in cardiothoracic surgery:

FROM MECHANISMS TO OUTCOMES

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Continuing insights into CABG-related SIRS

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For years, blood flow delivery and gas exchange have been primary foci for investigations of the pathophysiologic events that occur during cardiac surgery with cardiopulmonary bypass (CPB). More recently, the focus has shifted to include the inflammatory response, or more specifically, the systemic inflammatory response syndrome (SIRS), which is a nonlocalized whole-body response that potentially can affect all tissues and vital organs.

SIRS is the outcome of a complex immunologic reaction that includes activation of cellular, humoral, fibrinolytic, and hemostatic systems. CPB is increasingly recognized as an environment where both blood cells and chemical mediators of inflammation are

activated. Indeed, SIRS occurs in the majority of, if not all, patients undergoing cardiac surgery with CPB. Most recover without any lasting sequelae, but in some the consequence is substantial morbidity (eg, neurocognitive disorders, stroke, acute lung injury, multiple organ failure) and even death.¹

In the context of cardiac surgery, SIRS is precipitated by surgical trauma and contact activation. Contact activation is a phenomenon unique to CPB—as blood passes through the extracorporeal circuit and over the artificial membrane, coagulation factor XII (Hageman factor) is activated. Activated Factor XII, in turn, triggers a series of cascade systems involving the coagulation and fibrinolytic pathways, kinin release, and complement activation. Despite the varied natures of these systems, the end result is activation of formed blood elements, platelets and leukocytes in particular.

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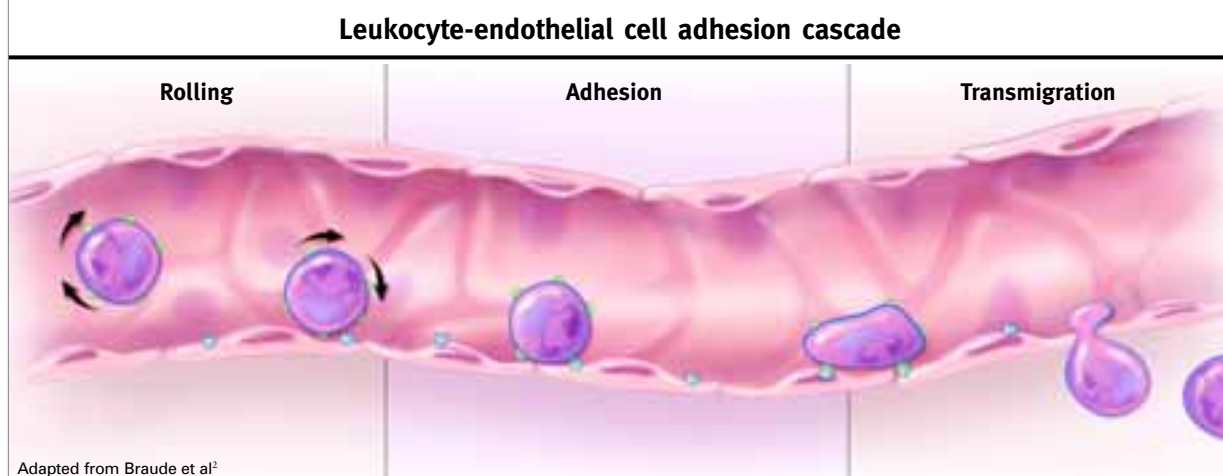


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FIGURE 1



■ CELL ADHESION CASCADE

Leukocytes, in conjunction with endothelial cells, play a major role in the evolution of SIRS. Leukocytes are activated by CPB-associated contact activation, as well as complement and various cytokines. Once activated, leukocytes express adhesion molecules such as L-selectins and integrins, which make the cell surface sticky. Endothelial cells are similarly activated by such factors as complement, endotoxin, tumor necrosis fac-

tor- α , and interleukin-1 (IL-1) and IL-6. Activated endothelial cells express corresponding adhesion molecule ligands such as E-selectins, intercellular adhesion molecules (ICAM), vascular cell adhesion molecules (VCAM), and IL-3.

During SIRS, activated leukocytes infiltrate the affected organ through a process known as leukocyte-endothelial cell adhesion cascade. Briefly, this cascade progresses in 3 stages—rolling, firm adhe-

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Intended audience: This educational activity is designed for practitioners who care for cardiac surgery patients, including cardiothoracic anesthesiologists, cardiothoracic surgeons, perfusionists, and others who desire to expand their knowledge of the medical and scientific information currently available.

Statement of need: Numerous pathologic processes occur during cardiothoracic surgery that requires cardiopulmonary bypass. Their most well-known clinical consequences include systemic inflammatory response syndrome, myocardial ischemia-reperfusion injury, and excessive postoperative bleeding. Efforts to minimize the insult secondary to surgery with consequent reduction of transfusion requirements and postoperative morbidity have been wide-ranging; this supplement focuses on prevention of inflammation and hemostatic abnormalities. It presents current investigative and clinical data as well as strategies for improving patient outcomes.

Learning objectives: Upon completion of this activity, participant should be able to discuss:

- The systemic inflammatory response syndrome as it occurs in patients undergoing coronary artery bypass grafting and cardiopulmonary bypass.
- The significance of platelet desensitization and inhibition of the protease-activated receptor-1 during cardiac surgery.
- The clinical efficacy of various methods investigated for the prevention of myocardial ischemia-reperfusion injury.
- The secondary effects of serine protease inhibition after cardiothoracic surgery.

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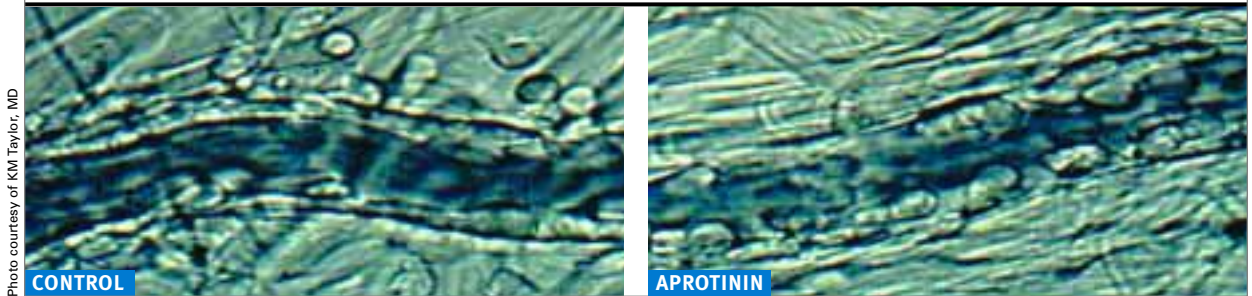
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FIGURE 2

Effect of aprotinin on inflammation-induced leukocyte transmigration



sion, and transmigration. Under normal conditions, leukocytes flow freely through blood vessels. When the inflammatory process is initiated, leukocytes migrate to the outer edges of the blood stream and roll along the vessel's surface endothelial cells in a slow, deliberate rolling motion (**Figure 1**). This process is mediated by selectins located on the leukocyte cell surface. Leukocyte rolling progresses quickly to firm adhesion to the vessel wall; this portion of the cascade is mediated by integrins. In the final step, transmigration, leukocytes migrate through the vessel wall to adjacent tissue, thereby bringing the inflammatory process into tissues and vital organs.

The leukocyte-endothelial cell adhesion cascade was demonstrated in the 1980's in a canine model of CPB.² Prior to surgery, a lung biopsy showed leukocytes freely flowing within the pulmonary blood vessels. However, after 90 minutes of CPB, many leukocytes became adherent to the pulmonary vascular endothelium, and by 120 minutes, they had moved through the pulmonary blood vessel wall across the alveolar capillary membrane into the lung airspace.

■ PREVENTION

Many avenues are being explored in our efforts to prevent or attenuate CPB-associated SIRS. Approaches include changes to the CPB circuit and surgical techniques and administration of pharmacologic agents to inhibit leukocyte activation, adhesion, or transmigration.

■ CIRCUIT MODIFICATION

Gourlay et al performed several in vitro studies to evaluate what effect CPB tubing, made of diethylhexyl phthalate (DEHP)-plasticized polyvinylchloride (PVC),

had on systemic inflammation. DEHP is a plasticizer that makes PVC tubing malleable. In the first study, which used a rat recirculation model, expression of the inflammatory marker CD11b increased as the surface area of DEHP PVC tubing increased. After 60 minutes of recirculation, expression of CD11b increased by 181% to 294% as the DEHP PVC surface area was increased, compared with 134% in control animals.³

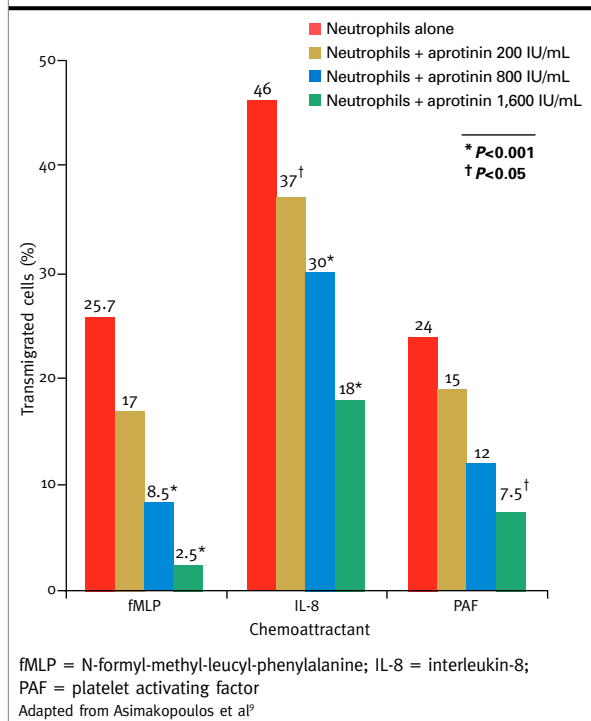
In a second study, again using the rat recirculation model, reducing the amount of DEHP on the surface of the PVC tubing with a methanol wash significantly reduced expression of CD11b. Following 60 minutes of recirculation, CD11b expression increased by 34% in the control group, by 97% in the methanol-washed group ($P < 0.001$ vs controls), and by 194% in the unwashed group ($P < 0.001$ vs controls and methanol-washed).⁴

Results were similar in a third study that assessed the inflammatory response of rat and human leukocytes to DEHP PVC. CD11b levels increased significantly as human and rodent blood concentration of DEHP were increased. At a DEHP concentration of 3.0 mg/L, the highest concentration evaluated, CD11b levels increased from baseline by 214% in human blood ($P < 0.001$) and by 237% in rat blood ($P < 0.001$). This effect was reduced after methanol washing; CD11b levels increased from baseline by 117% in human blood ($P > 0.1$) and 150% in rodent blood ($P > 0.1$).⁵

A fourth study evaluated the effect of hemodilution in the same recirculation model. In rats exposed to DEHP PVC tubing, CD11b levels increased by 189% when mean hematocrit was 31% and by 369% when mean hematocrit was 21%.⁶ In rats not exposed to DEHP PVC, CD11b levels increased by 110% at a hematocrit of 41% and by 127% at a hematocrit of 21%.

FIGURE 3

Effect of aprotinin on in vitro leukocyte transmigration



DRUG INTERVENTIONS

Pharmacologic interventions are also being evaluated for their ability to decrease the inflammatory response following surgery with CPB. They include monoclonal antibodies such as pexelizumab and other agents targeted against inflammatory mediators.

Pexelizumab inhibits the generation of C5a and C5b-9 complement products. Preclinical studies suggested that inhibition of C5a and C5b-9 complement products reduce inflammation, myocardial necrosis, and apoptosis.⁷ In the Pexelizumab for Reduction in Infarction and Mortality in Coronary Artery Bypass Graft (PRIMO-CABG) Surgery study, 3,099 patients undergoing CABG surgery with or without valve surgery were assigned randomly to pexelizumab (a bolus of 2.0 mg/kg 10 minutes before surgery followed by 0.05 mg/kg per hour for 24 hours) or placebo. The risk of death or myocardial infarction within the next 30 days was significantly reduced in all patients who received pexelizumab (n=1,553), or more specifically, in those who underwent CABG surgery with or without valve surgery (relative risk [RR] 0.82, 95% confidence interval [95% CI] 0.68-0.99; P=0.03). However, the 30-day risk of death or myocardial

infarction was not significantly reduced in patients who underwent CABG surgery alone (RR 0.82, 95% CI 0.66-1.02, P=0.07).⁷

CD163. Investigators have identified a potential new pathway for pharmacologic therapy; monocytes/macrophages that carry the CD163 receptor have been linked to anti-inflammatory activity. CD163 is a hemoglobin scavenger receptor that mediates endocytosis of hemoglobin-haptoglobin complexes. Binding of hemoglobin-haptoglobin complexes to CD163 monocytes triggers secretion of IL-10, which in turn, induces heme oxygenase-1 stress protein synthesis.⁸

Elevated CD163 levels have been detected on circulating monocytes of patients following CPB.⁸ The elevations occurred during the resolution phase of the inflammatory response—24 to 72 hours after CPB was initiated.

Aprotinin. Several studies have evaluated the effects of aprotinin on SIRS during CPB surgery. Asimakopoulos et al sought to determine whether aprotinin suppresses leukocyte activation in general or has specific actions during the endothelial cell-leukocyte adhesion cascade.⁹ The investigators used intravital microscopy, which allowed them to visualize directly leukocyte movement in the mesenteric microcirculation of anesthetized rats after applying an inflammatory stimulus with N-formyl-methyl-leucyl-phenylalanine (fMLP). Administration of aprotinin, at a dose comparable to clinically employed high-dose aprotinin, significantly inhibited the transmigration of leukocytes across the mesenteric vessel wall (**Figure 2**). Transmigration into surrounding tissues had decreased by 73% at 40 minutes (P<0.05 vs placebo), and the decrease was still significant at 60 minutes (P<0.05 vs placebo). Aprotinin did not affect leukocyte rolling or adhesion, the 2 other steps in the adhesion cascade.

In parallel experiments, aprotinin also inhibited leukocyte transmigration through cultured human endothelial cells in a dose-dependent manner in the presence of 3 leukocyte chemoattractants: fMLP, IL-8, and platelet activating factor (PAF) (**Figure 3**).⁹

CONCLUSION

The punitive effects of CPB-associated SIRS can lead to prolonged hospitalization following cardiac surgery. Neurocognitive disorders, stroke, acute lung injury, multiple organ failure, and death have all been reported.¹ Currently, researchers are evaluating vari-

ous strategies aimed at decreasing the inflammatory response that occurs during surgery with CPB. These strategies include changing the surface materials of the CPB circuit, making changes in surgical techniques, and administering pharmacologic agents. Results from an in vitro study⁹ and an in vivo rat model study⁹ suggest that aprotinin, administered at high doses, may decrease the inflammatory response associated with CPB. ■

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Platelet activation and clinical relevance of PAR inhibition

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Following surgery that requires cardiopulmonary bypass (CPB), excessive bleeding occurs in about 5% to 10% of patients. Upon reexploration, no surgical cause for bleeding is found in the majority of these cases, suggesting that CPB probably is associated with some degree of hemostatic impairment. Thrombin may contribute to the hemostatic defect by desensitizing platelets, a phenomenon also known as platelet exhaustion.

Thrombin, a serine protease, is generated in significant amounts during cardiac surgery that employs CPB but not during thoracic surgery performed without CPB.¹ A significant number of platelets are activated in the presence of the increased thrombin, as evidenced by the numerous small, degranulated platelets that can be observed after CPB. For exam-

ple, electron microscopic studies have shown a predominance of grade 4 platelets before CPB and of grade 1 (ie, exhausted) platelets after CPB.²

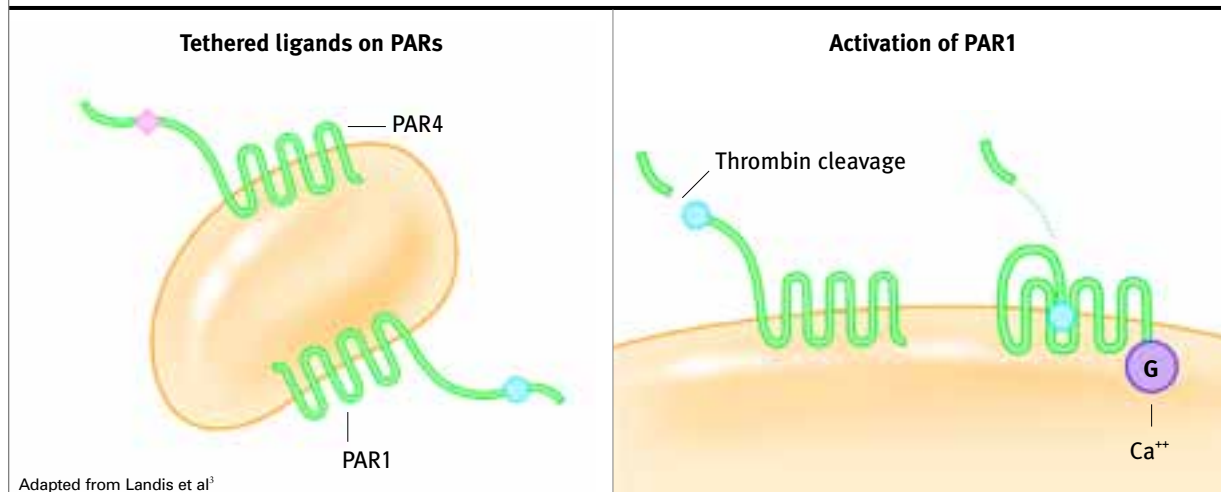
■ PAR1 RECEPTOR

The protease-activated receptor-1 (PAR1) is the principal thrombin receptor on platelets; it is a high-affinity receptor that can be activated at low levels of thrombin. PAR4, the second platelet thrombin receptor, is a low-affinity receptor that requires high thrombin concentrations for activation. The PARs are unique among cell surface receptors in that they carry their own ligand (ie, a tethered ligand) in their amino-terminal exodomain (**Figure 1**).³ Once thrombin binds to and cleaves the amino-terminal exodomain, the new terminus serves as a tethered ligand that docks intramolecularly within the body of the receptor to effect transmembrane signaling via cytoplasmic G-proteins. In contrast to reversible

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FIGURE 1

Activation of PARs



ligand binding, thrombin cleavage cannot be undone; once the previously hidden ligand is unmasked, the mechanisms for platelet desensitization are set into motion.

In vitro activation of PAR1 can be accomplished with a synthetic peptide chain called thrombin receptor agonist peptide-6 (TRAP-6). TRAP-6 mimics the last 6 amino acids (SFLLRN) of the PAR1 tethered ligand and can activate the PAR1 receptor without cleavage of the exodomain (**Figure 2**). Ferraris et al compared the number of TRAP-induced platelet-leukocyte conjugates formed ex vivo in whole blood samples taken from 79 patients before, immediately after, and 24 hours after CPB.⁴ This technique provides a measure of platelet PAR1 function, since prior activation by thrombin causes cleavage and loss of PAR1 from the platelet surface. TRAP-induced platelet activation was significantly reduced immediately following and up to 24 hours after CPB ($P=0.001$) in the 8 patients who required 10 or more units of transfused blood. These results suggest that increased blood loss following CPB results from decreased PAR1 responsiveness or platelet desensitization.

■ PLATELET PRESERVATION IN VITRO

Strategies aimed at limiting the effects of thrombin during CPB may help to decrease bleeding complications in patients undergoing cardiac surgery.

Indirect protection. Antifibrinolytic agents, such as tranexamic acid, ϵ -amino caproic acid, and aprotinin, are commonly used in cardiothoracic surgery to

prevent excessive bleeding. There is some evidence that the lysine analogs (ie. tranexamic and ϵ -amino caproic acid) provide indirect platelet protection by limiting the production of plasmin, which can activate platelets.⁵ Aprotinin, being a broad-spectrum serine protease inhibitor, also protects against the platelet activating properties of the serine protease plasmin.⁶ Compared to the lysine analogs, aprotinin consistently confers greater platelet preservation, resulting in reduced need for transfusion and less exposure to allogeneic blood products.⁷⁻⁹ Indeed, the serine protease inhibiting properties of aprotinin may play a direct role in preventing platelet desensitization during CPB.

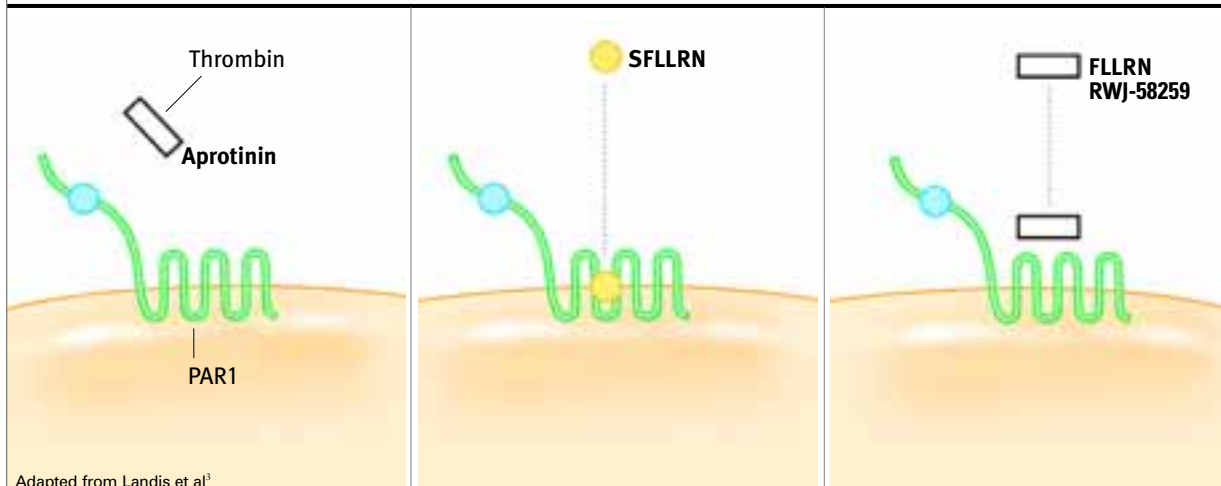
Direct protection. Aprotinin, but not tranexamic acid, has been shown to directly protect platelets through mechanisms that are independent of plasmin inhibition.^{10,11} In vitro studies have shown that the drug selectively blocks PAR1-dependent platelet activation by thrombin.¹²

To study the direct effects of aprotinin on platelet function, Shinfeld et al gave high-dose aprotinin (6×10^6 KIU) to 20 patients before and during CPB.² Platelet aggregation was significantly better ($P<0.05$) and 24-hour blood loss significantly less ($P<0.01$) than in 20 patients who received single-donor plateletpheresis concentrate immediately after CPB. Additionally, platelet aggregation was maintained at a pre-CPB grade 4 in 90% of patients who received aprotinin but in none of the patients who received plateletpheresis concentrate.

The conclusion by Poullis et al—that aprotinin

FIGURE 2

Drugs that act directly on PAR1



Adapted from Landis et al³

inhibits thrombin-induced platelet activation by preventing proteolysis of PAR1—was based on *in vitro* experiments in which aprotinin inhibited platelet aggregation in a dose-dependent manner.¹¹ At drug concentrations of 50, 100, and 160 KIU/mL, platelet aggregation was inhibited by a mean of 43%, 61%, and 87%, respectively. However, aprotinin did not inhibit platelet aggregation in the presence of TRAP-6, which activates PAR1 directly without proteolytic cleavage.

In addition to showing that the drug prevents thrombin cleavage of PAR1, other data from the Poullis study demonstrated that aprotinin had no effect on nonproteolytic mechanisms of platelet activation. Blockade of thrombin cleavage by aprotinin did not prevent subsequent aggregation induced by platelet agonists such as collagen or epinephrine, both of which are present in the chest cavity during surgical procedures. The results of the Poullis study therefore suggest that with respect to platelet function, aprotinin may be simultaneously antithrombotic and hemostatic; antithrombotic since it prevents platelet activation by thrombin and hemostatic since it preserves platelets and allows them to participate in the formation of hemostatic plugs at wound and suture sites.

FLLRN. FLLRN is the prototype of PAR1 antagonist peptides that are based on the tethered ligand's amino acid sequence.¹³ *In vitro* studies comparing FLLRN and aprotinin have elucidated how these agents confer platelet protection. Like aprotinin, FLLRN effectively inhibited thrombin-induced platelet aggregation. However, unlike aprotinin, FLLRN did not prevent cleavage of the PAR1 ligand

from the PAR1 receptor; instead, it blocked the ligand-binding site.

RWJ-58259. The selective PAR1 antagonist peptide RWJ-58259 is among new compounds being evaluated for antithrombotic potential. A synthetic peptidomimetic of the PAR1 tethered ligand sequence, RWJ-58259 competitively inhibits the PAR1 ligand-binding site. Administration of the small-molecule antagonist prevented thrombus formation and vascular occlusion in a monkey model of injury-induced thrombosis.¹⁴ Measurements of platelet aggregation showed complete inhibition of PAR1, but not of PAR4. Staining of mural thrombi indicated significantly reduced thrombus platelet deposition. Clinical trials of RWJ-58259 have not yet begun.

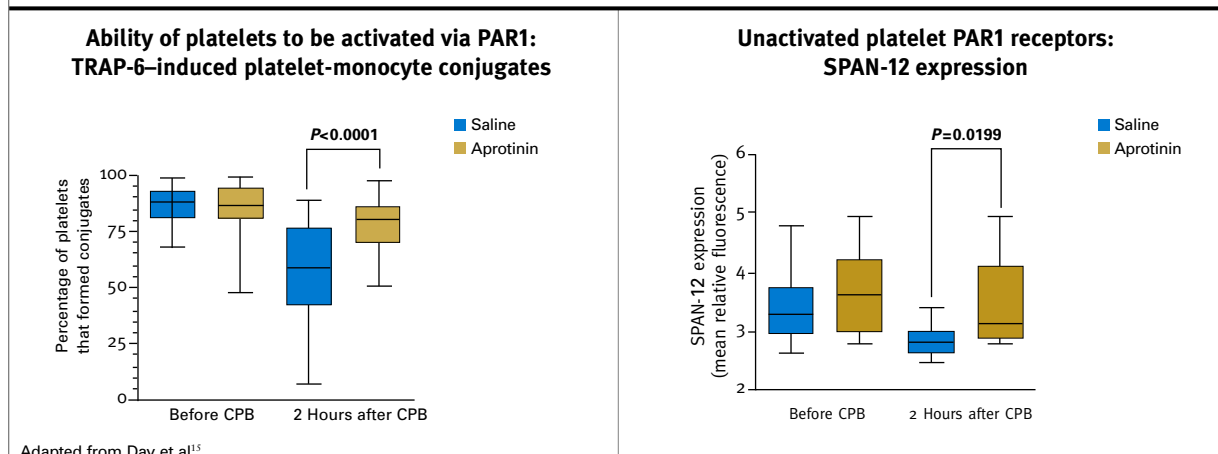
■ PLATELET PRESERVATION IN VIVO

My colleagues and I performed a randomized, placebo-controlled trial to determine whether aprotinin's *in vitro* PAR1-sparing effect also occurred clinically. Thirty patients undergoing coronary artery bypass grafting (CABG) and CPB were randomized to intravenous saline (n=17) or high-dose aprotinin (0.5×10^6 KIU) (n=13).¹⁵ Platelet PAR1 function was assessed before and after CPB by the technique of Ferraris⁴—this methodology measures the *ex vivo* formation of TRAP-6-induced platelet-monocyte conjugates.

Thrombin generation increased during CPB to levels similar to those seen in other placebo-controlled trials. In both the saline and aprotinin groups, the number of thrombin-antithrombin complexes

FIGURE 3

Effect of aprotinin on platelet PAR1 expression and function during CPB



increased within 5 minutes of starting CPB, peaked at the end of CPB, and then decreased to baseline over the next 5 days. The rate of thrombin decline was significantly more rapid in the aprotinin group at 24 hours ($P<0.001$) but not at any other time.

Two hours after CPB, platelets in saline-treated patients showed a significant loss of PAR1-specific function ($P<0.0001$) compared with the aprotinin group (Figure 3). Prior activation of PAR1 by thrombin was assessed using the monoclonal antibody SPAN-12, which recognizes only intact (ie, unactivated) receptors. Expression of SPAN-12 decreased significantly after CPB in platelets from the saline group ($P<0.02$), but remained essentially unchanged in the aprotinin group.

These results provide further evidence that aprotinin can preserve platelets during CPB. The platelet-sparing effect was most likely caused by direct targeting of PAR1 since thrombin generation did not differ significantly between the aprotinin and placebo groups for up to 2 hours after CPB. Moreover, supporting in vitro evidence showed that aprotinin inhibited PAR1-dependent but not PAR4-dependent platelet aggregation ($P<0.001$).

■ ISCHEMIC STROKE

Platelet exhaustion has also been noted after acute ischemic stroke.¹⁶ Platelets from patients with acute cerebrovascular ischemia exhibit significant cleavage and internalization of PAR1 and fail to respond to thrombin in vitro, indicating that high concentrations

of thrombin had been present earlier in vivo. Thus, the pathogenic events of CPB-related stroke and ischemic stroke may be the same.

A decreased incidence in CPB-related stroke has been observed in patients treated with aprotinin, suggesting that the PAR1 pathway of platelet activation may be therapeutically targeted in such patients.^{17,18} For example, in a randomized, placebo-controlled trial of 287 patients undergoing CABG and CPB, Levy et al reported none of the 6 strokes (incidence, 2.1%) occurred in patients who received high-dose aprotinin.¹⁷

■ CONCLUSIONS

Thrombin generation during surgery with CPB can cause platelet exhaustion via activation of PAR1. Administration of aprotinin both in vitro and in vivo preserves platelet function by inhibiting proteolytic activation of PAR1. However, aprotinin has no effect on nonproteolytic pathways of platelet activation, which may be important for hemostasis in the chest cavity, suggesting that the drug may have a subtle antithrombotic but hemostatic mechanism of action. ■

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Strategies for attenuating myocardial ischemia-reperfusion injury

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Coronary artery bypass grafting (CABG) with cardiopulmonary bypass (CPB) and cardioplegic arrest is generally an effective and safe technique for coronary revascularization. However, numerous pathologic processes occur during CPB, including immunologic reactions known to be associated with the systemic inflammatory response syndrome. In addition, cardioplegic arrest and CPB frequently are associated with myocardial ischemia-reperfusion injury, which further serves to increase postoperative morbidity and mortality.

Most ill effects from ischemia-reperfusion injury

are attributable to contractile dysfunction. The contractile abnormalities result in part from altered calcium handling after administration of the hyperkalemic cardioplegic solution (intracellular calcium stores are released and extracellular calcium enters the cytosol) as well as enzyme dysfunction and actin-myosin abnormalities. Other pathologic events in myocardial ischemia-reperfusion injury include coronary microvascular dysfunction (caused by the no-reflow phenomenon, vascular spasm, and edema) and myocardial damage and necrosis.

Since the advent of cardiac surgery, many efforts have been made to provide perioperative myocardial protection. Examples include hypothermia; cardioplegic solutions with high concentrations of potassium or magnesium; blood cardioplegia; cardioplegia

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FIGURE 1

Myocardial gene expression during CPB

Genes with ≥ 4 -fold increase in upregulation

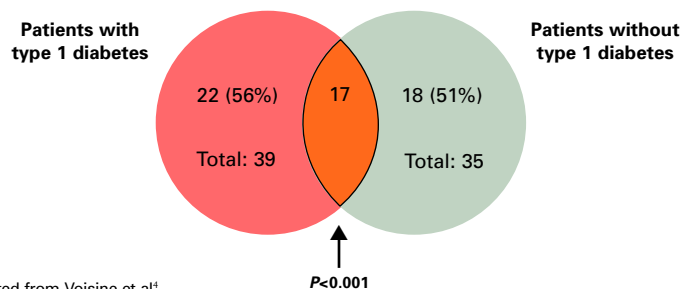
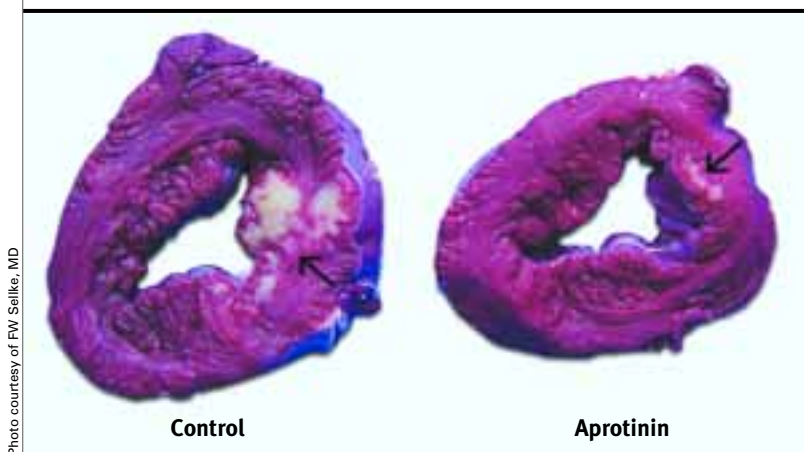


FIGURE 2

Effect of aprotinin on myocardial infarct size in pigs



additives such as lidocaine, adenosine, β -blockers, pinacidil, complement (C5a) inhibitors, antineutrophil agents, sodium-hydrogen exchange inhibitors, and poly (ADP-ribose) polymerase (PARP) inhibitors; gene therapy; and off-pump CABG. Some of these (eg, complement inhibitors, adenosine, antineutrophil agents, and sodium-hydrogen exchange inhibitors) were effective in laboratory models of cardioplegia and CPB surgery, but only blood cardioplegia has shown consistent clinical benefit by providing improved myocardial and vascular protection.^{1,2}

■ cDNA MICROARRAY TECHNOLOGY

Efforts to improve myocardial protection during CPB

would be facilitated by precise assessments of their efficacy. One such method, complementary DNA microarray technology, is a relatively new technique that only recently has been applied to the field of cardiac surgery.³

In essence, cDNA microarray technology permits screening of thousands of genes for differential expression. My colleagues and I used the technology to evaluate samples of atrial myocardium taken from 5 patients with type 1 diabetes and 5 matched, nondiabetic controls before and after CPB with hyperkalemia and blood cardioplegia.⁴ Total RNA was extracted from the tissue samples and reverse transcribed to cDNA, from which probes were prepared. Probe hybridization of the cDNA array was performed to identify altered gene regulation (ie, expression). In all, 12,625 genes were evaluated. Of these, 851 genes were upregulated in samples from patients with diabetes (vs 480 in control samples) and 443 were downregulated (vs 626 in control samples).

A >4 -fold increase in upregulation was detected in 39 genes from patients with diabetes and in 35 genes from controls (**Figure 1**). Seventeen of the highly upregulated genes were the same in both groups; hence, 22 of 39 genes and 18 of 35 genes were exclusive to each group. Highly upregulated genes found in myocardial samples of both groups included IL-6 (mediates leukocytosis, thrombosis, and lymphocyte activation), FOS (inflammatory and transcription activator), jun B (proapoptotic transcription factor), nuclear receptor subfamily 4-A1 (steroid receptor, regulates apoptosis), nuclear receptor subfamily 4-A2 (transcription coactivator, stimulated by prostaglandin E₂, interleukin-1B, and tumor necrosis factor), and dual-specificity phosphatase-1 and -5 (regulates cellular response to stress).

A >4 -fold decrease in downregulation was detected in 7 genes from patients with diabetes and in 16 genes from controls. None of the downregulated

genes were common to both groups.

While most of the changes in this study are predictable from previous studies, an examination of more than 12,000 genes simultaneously may uncover novel findings. These studies also demonstrate that technologies such as cDNA microarray methods may detect subtle changes in myocardial function and biochemistry that were previously unknown or not detected by conventional laboratory methods. Clearly, a goal of this work is to correlate patterns of altered gene expression in the myocardium and other tissues with clinical outcomes. An examination of clusters of inflammatory genes may help to improve our ability to evaluate various methods of myocardial protection, thereby lessening the inflammatory effects of CPB and improving postoperative cardiac function.

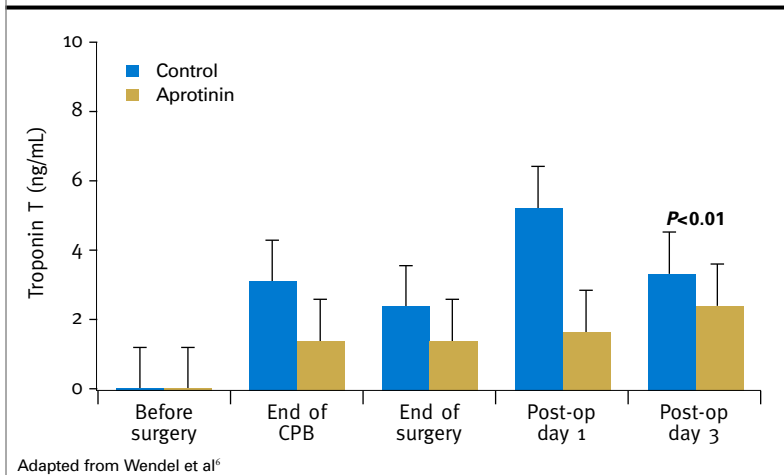
■ PROTECTION IN THE ANIMAL MYOCARDIUM

Recently Khan et al studied the myocardial protective effects of aprotinin in a porcine model of regional ischemia.⁵ After 30 minutes of regional myocardial ischemia, 12 pigs underwent 45 minutes of CPB and cardioplegia and 90 minutes of reperfusion. Six pigs received crystalloid cardioplegic solution and 6 received aprotinin (40,000 KIU/kg loading dose, 40,000 KIU/kg in the CPB pump prime, and 10,000 KIU/kg/hr infusion). Assessments included global myocardial function (left ventricular [LV] dP/dt), regional myocardial function (percent segmental shortening in the LV ischemic region), coronary blood flow (in the distal left anterior descending [LAD]), myocardial infarct size (staining of the distal LAD region), and infiltration of neutrophils and nitrogen radicals. In addition, coronary arteries 50 to 150 mm in diameter were removed from near the LAD, and their relaxation responses to adenosine diphosphate (ADP), substance P, and sodium nitroprusside were evaluated.

Global myocardial infarction and coronary blood flow were essentially the same in aprotinin and control animals. Regional myocardial infarction showed a trend toward improved function (less dyskinesia) in animals that received aprotinin. Infarct size was 40% smaller in pigs treated with aprotinin (Figure 2).

FIGURE 3

Effect of aprotinin on myocardial enzyme release



Myeloperoxidase levels, which are indicative of myocardial neutrophil infiltration, were markedly reduced in aprotinin-treated animals, as was nitrotyrosine staining (a measure of peroxynitrite). Microvascular function was better preserved in animals that received aprotinin; the microvessels of these animals showed significantly greater relaxation in response ADP and substance P. Measurement of vascular endothelial cadherin (a cellular adhesion molecule) expression showed that aprotinin-treated animals expressed more cadherin and thus were better able to preserve endothelial cell junctions.

Clinically, aprotinin-treated animals required less intraoperative fluids than controls to maintain the same hemodynamics. In fact, aprotinin-treated animals required 20% less fluid; the difference was significant and suggests that this agent may improve vascular integrity. Aprotinin-treated animals also had significantly less myocardial edema.

In summary, in this porcine model of regional myocardial ischemia, aprotinin improved regional myocardial function in the distal LAD region, reduced infarct size, prevented neutrophil accumulation and peroxynitrite generation, enhanced microvascular relaxation, increased endothelial cell expression of vascular endothelial cadherin, and decreased intravenous fluid requirements and myocardial edema.

■ PROTECTION IN THE HUMAN MYOCARDIUM

The myocardial protective effects of aprotinin also have been examined in clinical studies. Wendel et

al, in a randomized placebo-controlled trial of 40 patients undergoing CABG and CPB, looked at the effects of high-dose ($>4 \times 10^5$ KIU) aprotinin on serum levels of troponin T, which has a high specificity for myocardial ischemia and infarction, and more general indicators of ischemia, such as creatine kinase MB and lactate dehydrogenase.⁶ Three days after surgery, patients who received aprotinin had slightly but statistically significant lower levels of all three markers ($P<0.01$) (**Figure 3**). One could presume from these data that aprotinin protects the myocardium from perioperative ischemic injury; however, the biochemical differences did not correlate with clinical outcome.

Creatine kinase MB levels were significantly lower ($P=0.041$) after low-dose aprotinin in a study of 60 men undergoing CABG and CPB who were randomized to receive 280 mg of aprotinin or placebo in the CPB pump prime.⁷ There were no significant differences in perioperative levels of myeloperoxidase and interleukin-6, -8, and -10.

Several studies have shown aprotinin to reduce postoperative atrial fibrillation. In 1 of these, 120 patients undergoing coronary revascularization and CPB were randomized to full-dose aprotinin and leukocyte depletion by filtration or control.⁸ Atrial fibrillation occurred in 7.6% of the treatment group and 27% of the control group ($P<0.025$), a 72% reduction in incidence.

Aprotinin also may reduce the need for postoperative inotropic support. Clinical and hemodynamic variables were similar during the first 48 hours after CPB in 34 infants who underwent cardiac surgery; however, only 4 of those treated with full-dose aprotinin subsequently received enoximone (an investigational agent), compared with 10 in the placebo group ($P<0.05$).⁹ Moreover, aprotinin-treated patients needed significantly smaller doses of enoximone than did placebo patients. Similarly in a report by Mössinger et al of high-dose aprotinin versus control in 60 children who underwent cardiac surgery and CPB, aprotinin reduced the amount of chest-tube drainage

($P<0.05$), improved postoperative oxygenation ($P<0.05$), and reduced ventilator time ($P<0.05$).¹⁰

■ CONCLUSION

Many cardioplegia additives such as aprotinin, complement inhibitors, adenosine, antineutrophil agents, and sodium hydrogen exchange inhibitors have demonstrated benefit in animal models of CPB. To date in clinical studies, only blood cardioplegia has been shown to provide incremental myocardial protection over purely crystalloid solutions. The clinical benefits of these other agents, including the serine protease inhibitor aprotinin, still need to be determined. ■

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Examining outcomes in cardiothoracic surgery

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The serine protease inhibitor aprotinin is currently the only drug approved by the U.S. Food and Drug Administration to reduce blood transfusion in patients undergoing cardiopulmonary bypass (CPB) during coronary artery bypass grafting (CABG). Aprotinin has also been used in patients who undergo CPB during thoracic aortic aneurysm repair.* Unfortunately, there have been lingering concerns regarding adverse outcomes when aprotinin is used in these 2 surgical settings. Therefore, my colleagues and I undertook 2 studies—a meta-analysis of published reports and a case-controlled series—to evaluate the negative impact, if any, of aprotinin in patients undergoing CABG or thoracic aortic surgery.

CORONARY ARTERY BYPASS GRAFTING

The effect of aprotinin on clinical outcomes in CABG surgery has been evaluated in numerous clinical trials. However, several case series have suggested that the drug may have an adverse effect on such outcomes as mortality, myocardial infarction, renal failure, atrial fibrillation, and stroke. Clearly, only a rigorous review of the data can address any lingering doubts about the safety of this agent. To that end, Artyom Sedrakyan (Yale University), Tom Treasure (Guy's Hospital, London), and I searched the MEDLINE®, EMBASE®, and Pharm-line® data bases for randomized, controlled trials of aprotinin and CABG.¹ Additional references were identified from the reference lists of studies found in these data bases.

To be included in our meta-analysis, a study design had to: 1) include placebo control and randomized allocation to treatment; 2) enroll only patients undergoing CABG; 3) administer aprotinin continu-

ously during surgery; and 4) exclude other experimental drugs or devices.

Of 115 studies identified, 51 met the inclusion criteria. Outcomes of interest (ie, clinically significant endpoints) were reported in 35 trials, most of which were double-blinded and of high quality. The 3,879 patients enrolled in these 35 trials were generally men (83%) whose mean age was 61 years. Results from this meta-analysis suggest that the risk of death and renal failure were similar in the aprotinin and control groups (**Figures 1 and 2**). The risk of myocardial infarction was slightly decreased with aprotinin and that of stroke and blood transfusion significantly decreased.

Mortality. The mortality rate was 2.47% in the aprotinin group and 2.4% in the control group (relative risk [RR] 0.96, 95% confidence interval [CI] 0.65-1.40). The difference was not significant.

Renal failure. Similarly, aprotinin did not increase the risk of renal failure, which occurred in 1.48% of aprotinin-treated patients and in 1.28% of controls (RR 1.01, 95% CI 0.55-1.83).

Myocardial infarction. There was a trend towards a reduced risk of myocardial infarction with aprotinin. It occurred in 4.74% of aprotinin-treated patients and in 5.03% of controls (RR 0.85, 95% CI 0.63-1.14).

Stroke. Even more noteworthy was the significantly reduced risk of stroke seen in patients who received aprotinin. The incidence of stroke was 1.1% in the aprotinin group and 2.22% in the control group, which represents a risk reduction of 47% (RR 0.53, 95% CI 0.31-0.90). Thus, 10 fewer strokes can be expected in every 1,000 CABG patients who receive aprotinin (95% CI -20, 0).

Blood transfusion. Fewer patients treated with aprotinin needed blood transfusion than did control patients (40.3% vs 63.3%). Indeed, aprotinin reduced the risk of transfusion by 39% (RR 0.61, 95% CI 0.58-0.66). For every 1,000 CABG surgeries performed with aprotinin, 250 patients did not require transfusion (95% CI -280, -220).

In summary, results from this meta-analysis of 35 studies that enrolled almost 4,000 patients show

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**Off-label use of aprotinin*

FIGURE 1

Adverse outcomes during CABG with aprotinin

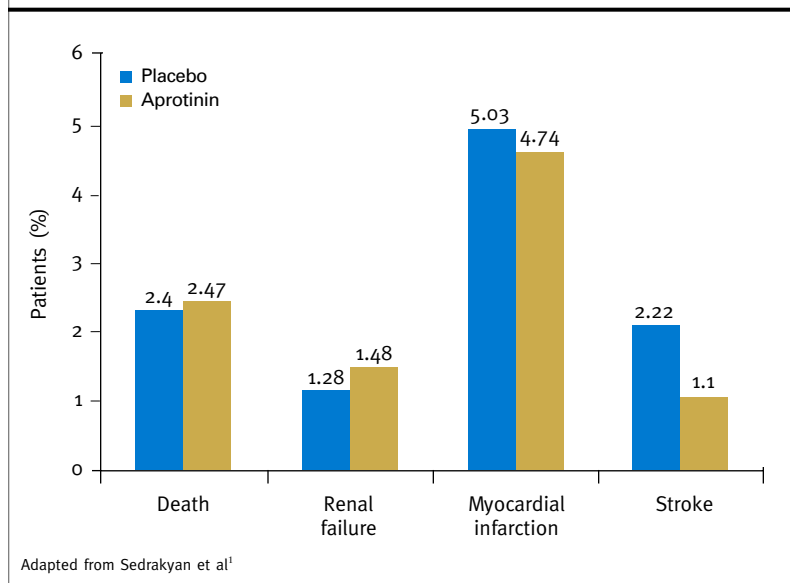
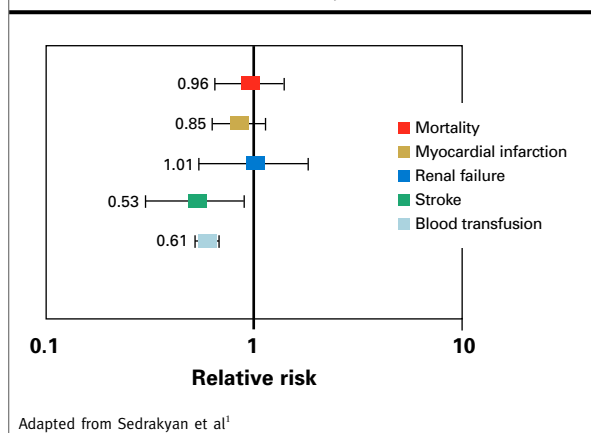


FIGURE 2

Relative risk of adverse outcomes during CABG with aprotinin



that compared with placebo, aprotinin does not exacerbate adverse outcomes; indeed, it appears to protect against stroke and to reduce the need for blood transfusion.

■ THORACIC AORTIC SURGERY

In contrast to the substantial literature on aprotinin and CABG, very little is known about the drug's impact on adverse outcomes in patients who undergo thoracic aortic surgery with CPB. Evidence initially suggested

that the protease inhibitor increased the risk of thrombotic events during deep hypothermic cardiac arrest. However, this effect was shown to be associated with inappropriate patient monitoring and not with aprotinin.

To further assess the effects of aprotinin in patients undergoing thoracic aortic surgery and CPB, my colleagues (Artyom Sedrakyan and Marianne Tranquilli) and I analyzed the data from a case-controlled series of 116 patients treated at our institution. This case-controlled study was conducted to evaluate the safety and effectiveness of aprotinin in patients undergoing thoracic aortic surgery. The results presented here are preliminary, pending analysis of larger patient cohorts.

Fifty-eight patients treated with aprotinin were matched to 58 control patients who received either aminocaproic acid or no hemostasis-enhancing medication. Cases were matched for age (mean, 62 years), body mass index (mean, 26 kg/m²), and sex (25 women in the aprotinin group and 23 in the control group). Aneurysm locations were also matched—in each group, approximately 84% were in the ascending aorta, 5% in the aortic arch, and 10% in the descending aorta. Approximately 40% of patients in each group had an aortic dissection, and 13% had preoperative shock. The mean time on CPB was 147 minutes for patients who received aprotinin and 135 minutes for the control group. Deep hypothermic cardiac arrest was used in 74% of patients who received aprotinin and 69% of control patients.

There were no differences between the 2 groups in terms of myocardial infarction, embolism, renal failure, and arrhythmia. However, patients treated with aprotinin had fewer strokes (2% vs 8%) and required less transfusion of red blood cells (mean, 2.5 vs 3.6 units). Aprotinin administration compared with control in fact decreased the incidence of stroke and red blood cell transfusion.

Overall, 28% of the aprotinin group and 43% of the control group experienced a complication (embolism, myocardial infarction, cerebrovascular accident, renal failure, pulmonary complication, arrhythmia, or death). Serious complications (myocardial infarction, cerebrovascular accident, or death) occurred in 6% and 16% of the 2 groups, respectively.

■ CONCLUSION

Our 2 studies of CPB during 2 different surgeries found no evidence of the putative toxicities associated with aprotinin. In fact, in both CABG and thoracic aortic aneurysm repair, treatment with aprotinin was associated with a decreased rate of stroke

and decreased transfusion requirements.¹ ■

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CME Post-Test

Please select the best answer to each of the following questions, and record it on the post-test answer form on page S16. Expiration date for credit: November 30, 2006.

1. **Complement inhibitors and antineutrophil agents have been shown to provide myocardial protection in both animal and clinical studies of cardiopulmonary bypass (CPB).**
 - a. True
 - b. False
2. **Aprotinin may decrease the inflammatory response associated with CPB by inhibiting**
 - a. Leukocyte rolling
 - b. Leukocyte adhesion
 - c. Leukocyte transmigration
 - d. None of the above
 - e. All of the above
3. **Which of the following agents is currently approved in the United States for reduction of blood loss and transfusions in patients undergoing coronary artery bypass grafting (CABG) and CPB?**
 - a. Tranexamic acid
 - b. Aminocaproic acid
 - c. Pexelizumab
 - d. None of the above
 - e. All of the above
4. **Which of the following agents prevent the protease-activated receptor-1 (PAR1) ligand from binding to its receptor?**
 - a. Aprotinin
 - b. FFLRN
 - c. RWJ-58259
 - d. None of the above
 - e. All of the above
5. **Strategies to prevent or alleviate the CPB-associated inflammatory response include**
 - a. Pharmacologic intervention
 - b. Improved surgical technique
 - c. Modification of CPB circuitry
 - d. All of the above
6. **Which of the following best describes the results of cDNA microarray studies of atrial tissue taken from patients with and without type 1 diabetes?**
 - a. No upregulated genes were common to both groups
 - b. All upregulated genes were common to both groups
 - c. No downregulated genes were common to both groups
 - d. All downregulated genes were common to both groups
7. **PAR1 carries its own ligand in the amino-terminal exodomain.**
 - a. True
 - b. False
8. **In a meta-analysis of 35 trials of CABG and CPB, significant adverse effects of aprotinin included increased risk of**
 - a. Myocardial infarction
 - b. Stroke
 - c. Renal Failure
 - d. None of the above
 - e. All of the above
9. **In a trial of 116 patients undergoing thoracic aortic surgery and CPB, significant adverse effects of aprotinin included increased risk of**
 - a. Myocardial infarction
 - b. Stroke
 - c. Renal Failure
 - d. None of the above
 - e. All of the above
10. **Aprotinin has antithrombotic and hemostatic properties.**
 - a. True
 - b. False

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