ⓒⓂⓔ Aprotinin in Cardiac Surgery: A Review of Conventional and Novel Mechanisms of Action

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Induction of the coagulation and inflammatory cascades can cause multiorgan dysfunction after cardiopulmonary bypass (CPB). In light of these observations, strategies that can stabilize the coagulation process as well as attenuate the inflammatory response during and after cardiac surgery are important. Aprotinin has effects on hemostasis. In addition, aprotinin may exert multiple biologically relevant effects in the context of cardiac surgery and CPB. For example, it decreases neutrophil and macrophage activation and chemotaxis, attenuates release and activation of proinflammatory cytokines, and reduces oxidative stress. Despite these perceived benefits, the routine use of aprotinin in cardiac surgery with CPB has been called into question. In this review, we examined this controversial drug by discussing the classical and novel pathways in which aprotinin may be operative in the context of cardiac surgery.

(Anesth Analg 2007;105:949–62)

In adult and pediatric cardiac surgery, the use of cardioplegic arrest and cardiopulmonary bypass (CPB) remains a common requirement. Although this approach allows for a quiescent and bloodless surgical field, myocardial reperfusion and separation from CPB is associated with hemostatic and hemodynamic sequelae that can complicate the early postoperative course (1–4). The mechanistic underpinnings for these post-CPB events include changes in the coagulation cascade intrinsic to the conduct of CPB, the evocation of a systemic inflammatory response, and tissue reperfusion injury. Changes in the coagulation cascade result in the consumption of coagulation factors necessary for maintenance of hemostasis, and thereby contribute to excessive bleeding in the early postoperative period (5,6). Induction of the coagulation and inflammatory cascades during CPB can exacerbate myocardial, pulmonary, neurologic, and renal dysfunction (7–11). In light of these observations, pharmacologic strategies that can prevent a derangement of the coagulation process, as well as attenuate the inflammatory reaction during and after cardiac surgery has been a continued area of clinical investigation. Aprotinin administration is one approach to accomplish this (12–19). Having been initially considered to act upon a specific pathway of the coagulation

cascade (20–21), aprotinin has been shown to exert multiple biologically relevant effects in the context of cardiac surgery and CPB (22–34).

Although there have been numerous reviews on the pharmacology and function of aprotinin concerning its effects on hemostasis (35–37), none has examined the potentially novel mechanisms of action of aprotinin in the context of cardiac surgery. Furthermore, in light of numerous controversial reports concerning aprotinin use, a greater understanding of the mechanisms of action of aprotinin is needed (38–45). Accordingly, the purpose of this review is three-fold: first, to briefly review the biochemistry and classical targets of aprotinin in the coagulation cascade, as this pathway affects other biologically relevant pathways involved in inflammation and ischemia–reperfusion (I/R) (Figure 1); second, to review relevant basic research on the novel effects of aprotinin and place these findings in context with the clinical studies that highlight these effects; third, to examine how past studies form a direction for future clinical and basic research regarding aprotinin in the context of cardiac surgery.

APROTININ: BIOCHEMISTRY AND CLASSICAL MECHANISMS OF ACTION

Aprotinin is a nonspecific serine protease inhibitor that has been used primarily as a hemostatic drug in cardiac surgery with CPB (46–54). Current dosing regimens were established around plasma concentrations of aprotinin needed to achieve the inhibition of numerous serine proteases in the coagulation cascade (Tables 1 and 2). Table 2 illustrates both the broad spectrum of proteases inhibited and the dose-dependent manner in which these proteases are inhibited. For example, plasma concentrations of 125 KIU (Kallikrein Inhibiting Units)/mL and 200 KIU/mL of
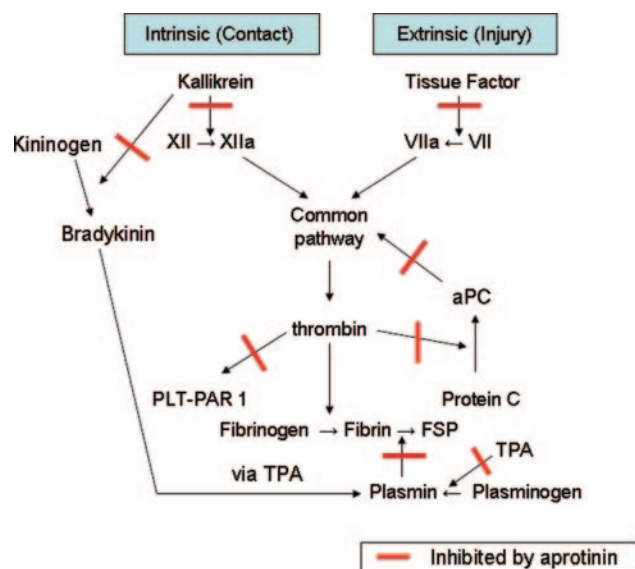
Figure 1. Broad-spectrum effects of aprotinin on multiple serine proteases in the coagulation and fibrinolytic cascades. (PLT-PAR-1 = protease-activated receptor-1; FSP = fibrin split products; TPA = tissue plasminogen activator).

Table 1. Traditional Dosing Regimens for Aprotinin

High dose (full Hammersmith): $5–6 \times 10^6$ KIU total	280 mg IV (2×10^6 KIU) bolus before sternotomy, then 70 mg/h (5×10^5 KIU/h) IV infusion, plus 280 mg (2×10^6 KIU) added to CPB pump prime
Low dose (half Hammersmith): $<5 \times 10^6$ KIU total	140 mg IV (1×10^6 KIU) bolus prior to sternotomy, then 35 mg/h (2.5×10^5 KIU/h) IV infusion, plus 140 mg (1×10^6 KIU) added to CPB pump prime
Pump prime only: 2×10^6 KIU	280 mg (2×10^6 KIU) added to CPB pump prime

IV = intravenous; CPB = cardiopulmonary bypass; KIU = Kallikrein Inhibiting Unit.

Table 2. *In-Vivo* Inhibition of Serine Proteases by Aprotinin

Serine protease	EC_{50} plasma concentration (KIU/mL)
PAR-1 receptor-thrombin	50–160
Plasmin	50–125
Neutrophil elastase	167
Activated protein C	66–214
Plasma Kallikrein	200–250
Thrombin	>1290
Tissue factor/factor VIIa complex	1430

KIU = Kallkrein Inhibiting Unit; EC_{50} = 50% inhibition of enzyme activity; PAR-1 = protease activated receptor-1.

aprotinin have been related to antifibrinolytic (antiplasmin) and antikallikrein activities, respectively (55–61).

CPB induces complex and widespread effects on the hemostatic system, which involves the activation and dysregulation of coagulation and fibrinolysis (1–10). The contact of patient blood with the artificial surfaces of the CPB circuit stimulates the intrinsic coagulation pathway through the activation of plasma kallikrein, factor XII, and factor XI (36,62). The extrinsic pathway is also stimulated during CPB because of surgical trauma and of monocyte activation with expression of tissue factor (63,64). The intrinsic and extrinsic pathways merge in the common final pathway to produce thrombin, which has several effects, including the conversion of protein C to activated protein C (aPC), activation of platelets via several receptors, and the generation of fibrin from fibrinogen (65). However, a major component of this entire hemostatic response induced by CPB is widespread fibrinolysis by plasmin, which is generated through fibrin formation as well as bradykinin-mediated activation of tissue-plasminogen activator (t-PA), which converts plasminogen to plasmin (66–68). Furthermore, there is platelet dysfunction due to activation by the CPB circuit, resulting in decreased expression of the surface glycoproteins Ib and IIb/IIIa, as well as increased activation of protease activated receptor-1 (PAR-1) by thrombin (3,16,69–71).

The major known targets of aprotinin within the hemostatic system are plasma kallikrein, plasmin, aPC, thrombin, PAR-1 on platelets, and tissue factor, as shown in Figure 1. Kallikrein, plasmin, aPC, and thrombin are all serine proteases, and have been thought to be directly inhibited by aprotinin binding to their active site and forming reversible enzyme-inhibitor complexes (8,36,65,68). It is generally accepted that a plasma concentration of >125 KIU/mL, which is achieved in the low-dose (half Hammersmith) regimen, is a plasma concentration at which aprotinin provides beneficial modulation of the hemostatic pathways, as plasmin is >90% inhibited at this concentration (35,58,72,73). PAR-1 and aPC are also inhibited by aprotinin concentrations that are commonly achieved in the low-dose regimen; but this has only been shown *in vitro*, as all *in vivo* studies on PAR-1 have been performed with the full Hammersmith dosing regimen (14,58,67,69). However, kallikrein and tissue factor are inhibited at aprotinin plasma concentrations >200 KIU/mL, which is reached after the initial bolus in the high- and low-dose regimens listed in Table 1, but is only consistently maintained throughout the CPB period by the high dose (full Hammersmith) protocol (59,60,63). The inhibition of kallikrein modulates the intrinsic coagulation pathway, which is mainly activated by the CPB circuit, whereas the inhibition of monocyte expression of tissue factor by aprotinin inhibits the extrinsic pathway, which is activated by surgical tissue trauma. These pathways merge into the common pathway, where aprotinin inhibits the actions of thrombin in a concentration-dependent manner (35,62,63,65). Thrombin will activate a wide range of platelet receptors, but it has extremely high affinity for the PAR-1 receptor, and thus the PAR-1 receptor activation is inhibited at a concentration that is reached in the low- and high-dose

Table 3. Systemic Effects of Aprotinin with Ischemia-Reperfusion

	Effect	References
Inflammation	Inhibits Kallikrein, plasmin, factor XIIa, complement	8
	Decrease expression of CD11b/CD18	76
	Reduce TNF-α, IL-8, and IL-6 release	31–34,77
	Reduces leukocyte transmigration	78
	Decreases ICAM-1/VCAM-1 expression	76
	Decreased neutrophil degranulation	79
Oxidative stress	Inhibits nitric oxide/nitrite production via NOS I/II and by reducing cytokine-induced nitrite production	33,80–84
	Reduces release of myeloperoxidase	85,86
Bioactive molecules	Inhibits Kallikrein and preserves capillary permeability	77
	Reduces C1, C3a, and C5a complement production	77,87–89

TNF = tumor necrosis factor, ICAM = intercellular adhesion molecule, VCAM = vascular cell adhesion molecule, NOS = nitric oxide synthase, IL = interleukin.

regimens, but it has only been investigated clinically with the high-dose regimen (69). It has recently been shown that aprotinin blocks the receptor cleavage step in the activation of PAR-1 by thrombin, which is separate from the serine protease catalytic domain of thrombin, illustrating another mechanism by which aprotinin may work (71). Finally, via direct inhibition of plasmin, and via the inhibition of kallikrein-mediated activation of t-PA, the fibrinolytic activity of plasmin is reduced in a dose-dependent manner by aprotinin (37,68,74). These effects of aprotinin result in the improved hemostasis demonstrated in multiple clinical trials (13,14,22,23,26,28).

POTENTIAL NOVEL EFFECTS OF APROTININ

Inflammatory Response to CPB and I/R Injury

Systemic and local inflammatory systems are activated by contact with the bypass circuit and by direct surgical trauma in the setting of CPB and I/R (1–7,75). Neutrophil activation is mediated by tumor necrosis factor-α (TNF-α), proinflammatory interleukins 6 and 8 (IL-6, IL-8), the complement system, factor XIIa, plasmin, and kallikrein (2,3). Once activated, leukocytes are directed to local areas of inflammation by chemoattractants, such as IL-8, and then transmigrate into tissues where they upregulate their production and release of proinflammatory cytokines, reactive oxygen species, elastases, peroxidases, and platelet activating factor, all of which cause tissue injury and dysfunction on both the intra- and extracellular levels (7,33). This inflammatory response remains one of the major causes of CPB-associated organ injury, resulting in increased morbidity and mortality (2). This review will examine the effects of aprotinin using both clinical and basic science reports. A general presentation of the role of aprotinin in attenuating inflammation, oxidative stress, and bioactive molecule release will be followed by a thorough review of the specific mechanisms by which aprotinin may effect heart, lung, kidney, and brain function in the setting of cardiac surgery with CPB, as summarized in Tables 3 and 4, respectively.

Inflammation and Aprotinin

During I/R, the ischemic myocardium itself becomes a specific source and target of inflammatory mediators. This inflammatory response results in clinical sequelae such as atrial fibrillation, myocardial edema, and left ventricular (LV) and pulmonary dysfunction (8). Aprotinin decreases the inflammatory response of both cellular (leukocytes) and noncellular (cytokines) components by inhibiting their activity in several ways, as shown in Figure 2. First, upstream inhibition of kallikrein, plasmin, factor XIIa, and complement can remove a major stimulus for widespread leukocyte activation (8). Second, aprotinin decreases the expression of leukocyte integrin CD11b/CD18 (76). Third, there is a dose-dependent antiinflammatory effect of aprotinin in patients undergoing cardiac surgery with CPB, including reduced TNF-α, IL-8, and IL-6 release, as well as inhibited neutrophil chemotaxis and monocyte activation (31,33,77).

Basic science research has provided interesting insights into the broad range of dose-dependent effects exerted by aprotinin on the cellular and noncellular components of the inflammatory response to myocardial I/R and CPB. It has been illustrated *in vitro* that aprotinin decreases leukocyte transmigration in response to IL-8; and in an intact rodent model, aprotinin reduces myocardial leukocyte infiltration (78). *In vitro* studies have shown that high-dose aprotinin decreases the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1, both of which are needed for the leukocyte to attach to the endothelium and then transmigrate (76). Leukocyte degranulation and release of proinflammatory and cytotoxic substances, such as neutrophil elastase, oxygen free radicals, TNF-α, and platelet activating factor, all of which lead to oxidative stress, is decreased by the use of aprotinin in high-dose animal protocols (79). Finally, the prototypical proinflammatory cytokine, TNF-α, also stimulates a variety of pathways that produce myocardial proteolysis, inducible nitric oxide generation, IL expression, and oxidative stress, all of which lead to LV dysfunction. Reduction in the generation and release of these substances is associated with an attenuation of I/R-mediated LV dysfunction (7,32–34). If *in vitro* and *in vivo* data of aprotinin use are considered as a whole, there is a dose-dependent reduction in the activation

Table 4. Novel Effects of Aprotinin on Major Organ Systems

	Effect	References
Heart	Decreases post-CPB and permanent atrial fibrillation	90,91
	Reduces infarct size, suppresses transient loss of contractility, improves wall thickening	92–94
	Preserves vascular-endothelial barrier via p38 MAPK pathway	95
	Synergistic with Na^+/H^+ pump inhibition to preserve cardiac function	96
	Inhibition of reperfusion-induced cardiac myocyte apoptosis	17
	Preserves ATP stores and reduces TNF-α after cardioplegia	32
	Reduces intramyocardial MPO release independent of TNF-α release	86
	Completely attenuates anesthetic preconditioning by sevoflurane	97
Lungs	Decreases extravascular lung water, improves A-ao$_2$ gradient, and lowers PVR	98–101
	Improves compliance by decreasing neutrophil sequestration	101–103
	Reduces MDA oxidative stress and preserves antioxidant activity which correlated with improved FEV1/Paco$_2$ values	104,105
Kidneys	No effect on RBF, FeNa, GFR, or renal prostaglandin release	106
	Causes renal tubular overload without tubular damage	107
	Increase urinary NGAL levels	108,109
	Reduces rise in serum creatinine and apoptosis in renal I/R	110
	Reduces NOS expression in isolated renal I/R	111
Brain	Half-Hammersmith had no effect on NCD, neuron specific enolase, or tau protein	112
	Full Hammersmith reduced NCD at 4 d/6 wk postoperative	113
	No neuroprotection from single bolus before cerebral ischemia	114
	Reduces neuron death and abnormal locomotor activity when given 4 h after cerebral ischemia	115
	Reduces neuron cell death via plasmin and MMP inhibition	115,116
	Inhibits S100β production and gliosis	117
	Reduces ICAM-1 expression, reduces leukocyte rolling and adhesion, and improves functional capillary flow density	118

A-ao$_2$ = lveolar-arterial oxygen; CPB = cardiopulmonary bypass; ATP = adenosine triphosphate; TNF = tumor necrosis factor; MPO = myeloperoxidase; PVR = pulmonary vascular resistance; MDA = malondialdehyde; FEV = forced expiratory volume; RBF = renal blood flow; FeNa = fractional excretion of sodium; GFR = glomerular filtration rate; MAPK = mitogen-activated protein kinase; NGAL = neutrophil gelatinase-associated lipocalin; I/R = ischemia-reperfusion; NOS = nitric oxide synthase; NCD = neurocognitive dysfunction; ICAM = intercellular adhesion molecule.
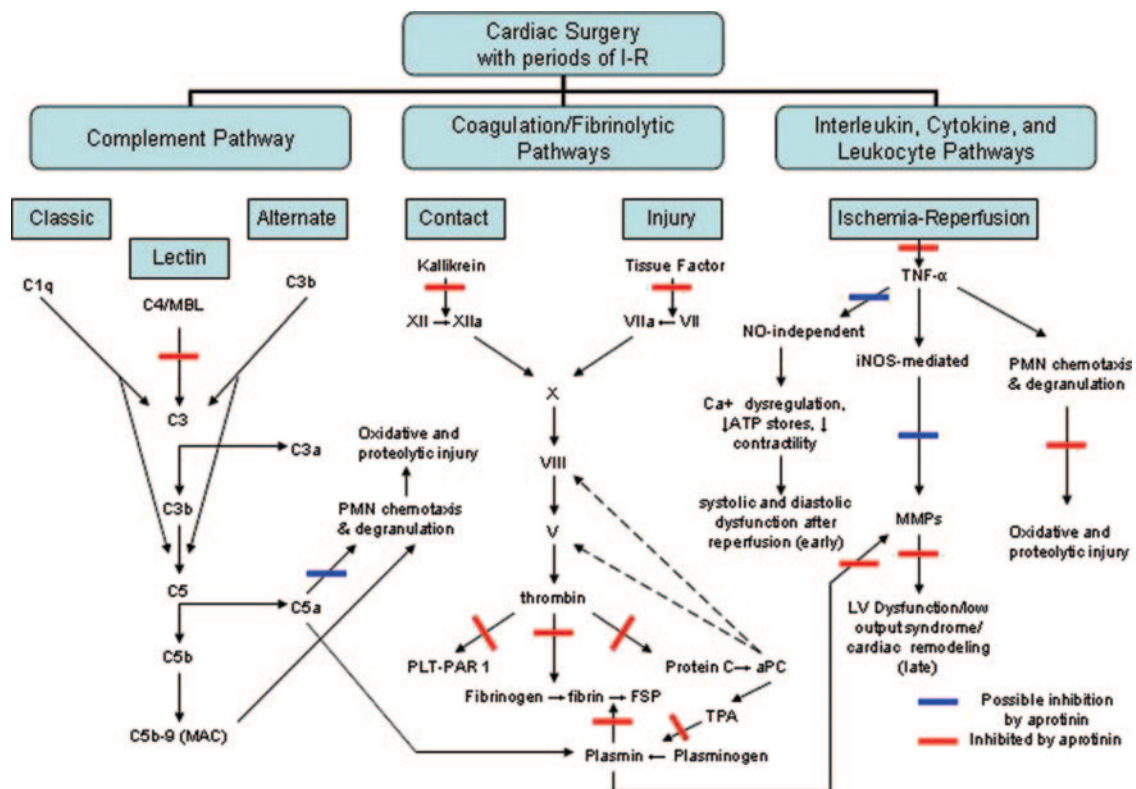

Figure 2. Complex interaction of the hemostatic, complement, and cytokine pathways in the setting of ischemia–reperfusion (I/R). (PLT-PAR-1 = protease-activated receptor-1; FSP = fibrin split products; TPA = tissue plasminogen activator; MMP = matrix metalloproteinases).

of the inflammatory response after CPB with myocardial I/R, with the greatest effects being seen at doses 2–4 times that of the high-dose regimen (71,79,119). Additional research is needed to elucidate the downstream mechanisms by which these cells and cytokines trigger myocardial dysfunction within the cell and extracellular matrix and the potential pathways by which aprotinin modulates this response to I/R, as shown in Figure 2. Furthermore, it is possible that dosing regimens higher than those currently used

clinically are needed to modulate other proteolytic pathways activated by CPB that affect both the cellular and noncellular components of inflammation; and since many of the antiinflammatory effects of aprotinin only last several hours, it is possible that dosing regimens extending into the postoperative period may be of benefit (40,71,79).

Oxidative Stress and Aprotinin

The concept of oxidative stress and free-radical injury of the myocardium and other tissue beds during reperfusion is based on several observations. First, after an ischemic period, reinstitution of normal blood flow induces myocardial dysfunction (120). Second, this restoration of blood flow to the ischemic area results in excessive production of reactive oxygen and nitrogen species (121). Third, antioxidant therapies and free-radical scavengers can diminish myocardial injury and dysfunction (120). Within cardiac and pulmonary tissue, the sources of oxidative stress are fibroblasts, cardiac myocytes, endothelial cells, and neutrophils.

Aprotinin has been shown to decrease pulmonary and cardiac oxidative stress related to CPB. Aprotinin reduces lung injury by inhibiting the production of nitric oxide *in vivo* and by inhibiting cytokine-induced nitrite production *in vitro* (122). Animal models have shown that aprotinin reduces nitrite accumulation and inducible nitric oxide synthase activity in the coronary vasculature of rats (80–83). Aprotinin is a direct inhibitor of nitric oxide synthase I and II; however, several studies have shown that aprotinin also indirectly reduces nitric oxide production by inhibiting TNF-α (33,122,84). Furthermore, as aprotinin reduces neutrophil transmigration, it also reduces the load of myeloperoxidase (MPO) released into the ischemic and reperfused myocardium (85,86). This benefit preserves contractile function after I/R and reduces infarct scar size, the latter of which was related to reduced oxidative stress, as measured by decreased MPO activity (34,92). Finally, aprotinin reduces cardiac reperfusion injury after I/R by affecting a decreased expression of proinflammatory genes and subsequent inhibition of polymorphonuclear neutrophils accumulation, leading to reduced intracardiac MPO levels (123).

Bioactive Molecules and Aprotinin

Amplification of bradykinin can have deleterious effects on LV function and hemodynamics after CPB. Bradykinin amplification leads to an increase in capillary permeability, with sequestration of fluid in tissues, and a decrease in systemic vascular resistance and cardiac output. By inhibiting kallikrein, aprotinin reduces the formation of bradykinin, resulting in a smaller decrease in systemic vascular resistance, as well as a reduction in capillary permeability (77). Also of note is that bradykinin is a major activator of the complement system. Furthermore, upstream aprotinin

inhibition of bradykinin activation results in a decreased production of complement proteins 3a and 5a, which activate leukocytes and further increase inflammation by causing their degranulation, as shown in Figure 2 (87). Aprotinin also inhibits complement protein 5a, which is a potent chemoattractant and vasoactive molecule that promotes neutrophil aggregation, adhesion, and degranulation (88,89). During CPB in animal models, a high-dose aprotinin regimen significantly reduced formation of complexes between complement protein 1 and its inhibitor, suggesting that aprotinin effectively reduced complement protein 1 plasma content (77). Although these results remain to be duplicated in clinical studies, they may correlate with some findings of a reduction in ionotropic support and improved pulmonary function in aprotinin-treated patients (41,83,124).

APROTININ AND ORGAN SYSTEMS

There is substantial clinical and basic science research investigating the effects of aprotinin on specific organs. The heart, lungs, kidneys, and brain can sustain injury during cardiac surgery with CPB that can lead to significant postoperative morbidity. Therapies to interrupt these injurious processes would be of profound clinical benefit, and recent findings are elucidating the specific effects of aprotinin in each of these systems.

Heart

Over the past 20 years, studies have consistently shown that aprotinin reduces intra- and postoperative bleeding and blood transfusion requirements when compared with placebo (20–23,26–28). However, the question of whether or not, and to what extent, aprotinin affects intrinsic myocardial performance in the setting of cardiac surgery with CPB has more recently been a topic of research. The IMAGE trial demonstrated no overall difference in myocardial infarction rates between aprotinin and placebo after risk factor adjustment (although the study did show increased graft occlusion in the Danish and Israeli sites, the cause of which is unknown) (125). However, the observational study by Mangano et al. (38) has recently reported a significant increase in myocardial infarction (48%) and heart failure (109%) rates in all aprotinin-treated patients versus control. Aprotinin has been reported to reduce the incidence of atrial fibrillation, which may suggest an indirect effect of aprotinin on the inflammatory cascade after CPB (90). Linking clinical outcomes with modulation of the inflammatory response, a prospective, randomized trial has shown that leukocyte depletion filters combined with full-dose aprotinin reduced the incidence of post-CPB atrial fibrillation by 72% (27% in control versus 7.6% in treated patients) (90). Two recent retrospective studies of approximately 2000 patients undergoing CPB for coronary artery bypass grafting

(CABG) or thoracic aortic surgery also found significant reductions in permanent atrial arrhythmias (91) and antiarrhythmic use in aprotinin-treated patients versus controls (41). In addition, aprotinin has been shown to block extracellular matrix degradation by inhibiting plasmin and matrix metalloproteinase (MMP)-2 activity in human smooth muscle cells (126). These findings suggest that aprotinin can affect extracellular and vascular remodeling through plasmin-mediated pathways. This may give insight into a mechanism by which aprotinin could inhibit postoperative atrial fibrillation in the cardiac surgical patient, as atrial fibrillation has been shown to be related to collagen deposition and cardiac remodeling in congestive heart failure (127), and the incidence of postoperative atrial fibrillation has been shown to be reduced with a reduced inflammatory state and decreased transfusion (128,129), both of which are affected by aprotinin.

Although these findings need to be validated with further prospective, randomized clinical trials, there is substantial basic science emerging that illustrates possible novel mechanisms by which aprotinin may exert such beneficial effects. In animal models of myocardial I/R, aprotinin use decreases the incidence of ventricular arrhythmias, preserves vascular-endothelial function and integrity, reduces infarct size, suppresses transient loss of contractility, improves wall thickening, and preserves a more complete coronary relaxation to bradykinin when compared with controls (92–96). Several novel mechanisms that are affected by aprotinin and may improve myocardial performance after I/R involve the p38 mitogen-activated protein kinase (MAPK) pathways, the activation of endogenous myocardial macrophages and transmigrating neutrophils, and the suppression of TNF-α activity with subsequent preservation of biochemical function. Khan et al. (95) have shown in pigs that aprotinin use preserves adherens junctions after regional I/R through the p38 MAPK pathway, resulting in preservation of the vascular-endothelial barrier and reduced myocardial tissue edema. The dose used in this study is 1.5–2 times larger than the full Hammersmith dose. Pruefer et al. (17) have shown in rats that aprotinin use exerts cardioprotective effects, as measured by lower oxidative stress and lower creatine kinase release, through inhibition of neutrophil-induced myocardial injury and inhibition of reperfusion-induced apoptosis of cardiac myocytes. The aprotinin dose used in this study roughly mimics the half Hammersmith dosing protocol. Furthermore, Bull et al. (32) have shown in myocardium stored in cardioplegic solution with aprotinin (200 KIU/mL) that there is increased adenosine triphosphate content and protein synthesis, a decrease in intramyocardial generation of TNF-α, and a decrease in the uptake of TNF-α into the myocardium compared with control. Of additional interest, a recent study has shown that improved myocardial systolic function after I/R is related to reduced MPO activity in the acute reperfusion period, but not to the suppression of TNF-α release (86). Finally, it has been reported from an *in vivo* rat model of myocardial I/R (25 min/2 h) that aprotinin completely blocks the anesthetic preconditioning effects of sevoflurane, as measured by infarct size (97). The mechanism of this final effect is unknown, but if this were true in humans, it would have profound clinical significance.

Lungs

There is a large body of work emerging concerning the optimal conditions for lung protection and the prevention of I/R lung injury in cardiac surgery with CPB and in lung transplantation with CPB. A recent retrospective study involving patients undergoing thoracic aortic surgery with CPB has shown that the full Hammersmith aprotinin dose was associated with reduced ventilation time and pulmonary complications when compared with controls (91). A similar study in mitral valve replacement surgery has shown that a low dose of aprotinin (20,000 KIU/kg) given at the start of CPB significantly decreased extravascular lung water and improved the alveolar-arterial oxygen (A-ao$_2$) gradient at 1-h and 24-h after surgery (98,99). Several other clinical trials have examined the mechanisms by which this improved pulmonary performance may occur. Ege et al. (102) found that low-dose aprotinin (15,000 KIU/kg) improved pulmonary compliance and the A-ao$_2$ gradient, which was correlated with reduced neutrophil sequestration in the pulmonary vasculature, thus reducing the cellular load which normally secretes reactive oxygen species, neutrophil elastase, and other proteolytic enzymes in reperfusion injury (30,99,130). In CABG surgery with CPB, Rahman et al. (19) reported an improved A-ao$_2$ gradient in patients receiving low-dose aprotinin (2 × 10^6 KIU) versus controls, which correlated with: 1) an attenuation of oxidative stress, as measured by reduced malondialdehyde (MDA), 2) preserved antioxidant activity, as measured by glutathione peroxidase activity, and 3) less neutrophil accumulation, all being measured in lung tissue samples obtained 5 min after cross-clamp removal. Erdogan et al. (104) investigated the effects of low-dose aprotinin (15,000 KIU/min) administered in the pulmonary artery on reperfusion injury in the setting of CABG surgery with CPB and similarly found better postoperative forced expiratory volume in 1 min and Paco$_2$ values in aprotinin-treated patients versus controls, which correlated with decreased levels of MDA and complement C4.

Basic science studies on the effects of aprotinin on pulmonary I/R injury in the setting of cardiac I/R have yielded similar results to those in clinical studies with respect to accumulating less lung water, decreasing pulmonary vascular resistance, and attenuating histologic inflammatory changes in lung parenchyma (93,100). In addition to this, animal models have shown that aprotinin has beneficial effects in isolated

lung injury models. Nader et al. (105) demonstrated in a rat model of pulmonary aspiration of gastric acid that low-dose aprotinin (10,000 KIU/kg) preserved the antioxidant activity of superoxide dismutase, but did not decrease lung injury, as measured by oxygenation, pulmonary MPO activity, and inflammatory cytokine levels, including TNF-α, IL-β, IL-10, and interferon-γ. Alternatively, in an *in vivo* rabbit model of isolated lung I/R, aprotinin (150 KIU/mL in lung protection solution) preserved oxygenation and reduced the A-ao$_2$ gradient when compared with controls (101). As with other studies, in aprotinin-treated subjects, these effects were correlated with reduced levels of MDA in lung tissue samples and a lower percentage of neutrophils in bronchoalveolar lavage fluid. On morphologic analysis, the aprotinin-treated animals had smaller percentages of pathological lesions and lower grades of alveolar hemorrhage than controls.

Koksal et al. (103) recently demonstrated that aprotinin is protective against pulmonary dysfunction in a model of lower limb I/R (4 h/1 h) in rats. Aprotinin exerted more of an antioxidant effect than calcium dobesilate or *N*-acetylcysteine. Aprotinin reduced peribronchial and interstitial accumulation of neutrophils compared with controls (103). These studies would suggest that pulmonary I/R injury is reduced by aprotinin through a reduction in neutrophil accumulation and attenuation of the inflammatory response, leading to decreased oxidative stress, a preservation of antioxidant capacity, and a preserved vascular-endothelial integrity which, together, culminate in a more normal lung parenchyma with preserved function. Furthermore, since these findings occurred in the setting of cardiac, pulmonary, or lower limb I/R, but not with chemical injury from aspiration, aprotinin may have benefits specific to the pathways activated in I/R (101,103,105).

Kidneys

Acute renal failure after cardiac surgery is a serious and common complication associated with a high mortality rate (131,132). Only one prospective, randomized, placebo-controlled trial with more than 100 patients has investigated the effects of aprotinin with postoperative renal function as the primary outcome (133). This study demonstrated no significant difference between aprotinin-treated patients and controls with respect to creatinine, electrolytes, blood urea nitrogen, urinalysis, or abnormal creatinine clearance rates, except on postoperative day 7, when there was a transient increase in creatinine levels in the aprotinin-treated patients. In a *post hoc* analysis of adverse events, D'Ambra et al. (134) did show a significant increase in renal dysfunction in aprotinin-treated patients. However, this was not dose-related, and diabetes mellitus was noted as a confounding variable.

In two observational reports with propensity-scoring, Mangano et al. (38) and Karkouti et al. (39) have shown significant increases of renal dysfunction

in aprotinin-treated patients versus controls. Augoustides et al. (132) have recently reported, from a large retrospective, observational study, that aprotinin use was a predictor for renal dysfunction after thoracic aortic surgery with deep hypothermic circulatory arrest when compared with aminocaproic acid. A recent retrospective report of prospectively gathered data (Merged Cardiac Registry) from 11,198 cardiac surgical patients suggested that the increase in renal failure seen in patients who were administered aprotinin was related to increased transfusions seen in that high-risk population and not to aprotinin use (135). Transfusions may account for the findings of these retrospective reports where there was no randomization for aprotinin use and aprotinin was often given to the population at higher risk for bleeding.

In light of this, clinical and basic science research has begun to focus on some of the mechanisms by which aprotinin affects the kidney in the setting of cardiac surgery with CPB, particularly subclinical proximal tubule dysfunction. Aprotinin is taken up by the proximal renal tubules in the kidney and much of it remains in the epithelial cells of the proximal tubule for more than 24 h after administration, and possibly several days (136,137). Schweizer et al. (106) demonstrated that full Hammersmith aprotinin dosing had no effect on renal plasma flow, fractional excretion of sodium, or glomerular filtration rate when compared with controls. This study further reported that aprotinin had no effect on renal release of vasodilatory prostaglandins (6-keto-PGF1α), possibly alleviating some of the concern about the interaction of angiotensin converting enzyme inhibitors with aprotinin, and illustrating a mechanism by which aprotinin may not affect the renal system.

Fauli et al. (107) found, in a prospective, randomized study of elective CABG patients with normal baseline renal function, that the full Hammersmith dose of aprotinin correlates with transient renal tubular overload without tubular damage when compared to the pump prime only dose and controls. In this study, clinical and subclinical renal function tests (creatinine, α_1-microglobulin, β-glucosaminidase) were followed from the preoperative period to 40 days after surgery. α_1-Microglobulin is a marker for subclinical renal dysfunction that correlates with a reduced capacity for tubular reabsorption by the epithelial cells of the proximal renal tubule, even when no histologic change is observed (138). β-Glucosaminidase is a kidney-specific enzyme that is a sensitive marker for subclinical renal tubular injury, indicating lysosomal tubular damage. Aprotinin caused a significant increase in α_1-microglobulin excretion, but not β-glucosaminidase, indicating renal tubular overload without tubular damage. This effect persisted for only 24 h after surgery in patients treated with full Hammersmith aprotinin dosing. Of note, there was no difference between treated patients and controls in plasma creatinine at any time point (107).

Wagener et al. (108,109) further elucidated a mechanism of the interaction of aprotinin with the proximal tubule in the setting of CPB. Neutrophil gelatinase-associated lipocalin (NGAL) is a carrier protein in the kidney, the up-regulation of which occurs rapidly (1–3 h) after renal epithelial injury (139). NGAL up-regulation has been shown to occur in both animal models and in cardiac surgical patients after CPB (140,141). In a prospective trial of adult cardiac surgical patients, Wagener et al. (108) recently reported that urinary NGAL is highly correlated with acute, postoperative renal dysfunction, defined as a >50% increase in serum creatinine from baseline. In a further investigation, aprotinin was associated with higher levels of urinary NGAL after CPB compared with controls, but the rate of renal dysfunction was not evaluated as an outcome (109). Interestingly, the urinary NGAL levels at 3-h post-CPB in the aprotinin-treated patients in this study were 60% lower than those that correlated with renal dysfunction in the previous study (108). However, it is clear that NGAL is a reliable, early marker of renal dysfunction after renal injury in the setting of CPB. What remains to be demonstrated is the source of the NGAL in this setting, neutrophils, macrophages, or renal epithelium (139), the knowledge of which would be a crucial piece in clarifying the potential drug target and mechanism by which aprotinin may affect renal physiology after CPB.

The clinical studies mentioned above were all performed in the setting of cardiac surgery with CPB. In comparison, two animal studies are of interest. The first determined the effects of aprotinin on 1) renal function, 2) apoptosis and apoptotic signaling, and 3) the inflammatory response of the kidney in an rat model of isolated renal I/R (1 h ischemia) (110). The rats undergoing ischemia received saline solution alone or a higher dose of aprotinin than that used clinically (60,000 KIU/kg). Serum creatinine was evaluated, and the kidney was analyzed for expression of TNF-α, IL-1β, and IL-6, as well as the activation of p38 MAPK, caspase 3, caspase 8, and for apoptosis. Aprotinin decreased the rise in serum creatinine and apoptosis caused by renal I/R, reduced IL-1 β and IL-6 messenger RNA production, reduced caspase 8 activation, and showed a trend toward reducing TNF-α messenger RNA production after ischemia and decreasing p38 MAPK activation after 1 h of reperfusion. These results suggest that aprotinin provides protection from renal I/R injury, and that this may be done by affecting apoptotic signaling and inflammatory cytokine production (110). Ozer et al. (111) found similar results concerning the salutary effects of high-dose aprotinin in a rabbit model of isolated left renal I/R. Immunohistochemical analysis revealed that inducible nitric oxide synthetase expression was less intense in the aprotinin group, and the staining results for the aprotinin groups did not differ much from the nonischemic kidney within the same animal, suggesting that aprotinin may be beneficial in the prevention of systemic inflammation after transient renal ischemia (111). Whether the effects of aprotinin on these novel mechanisms are applicable to cardiac surgery with CPB remains to be elucidated, as these models concern direct renal ischemia and then reperfusion, whereas clinical research has been performed in the setting where the glomerular filtration rate is unchanged and effective renal plasma flow actually increases during CPB (107).

Brain

Neurocognitive dysfunction (NCD) is a common complication after cardiac surgery with CPB. Several recent retropsective reports suggest that the rate of central nervous system complications are increased in patients treated with aprotinin (38,39). However, the results from patients enrolled in prospective trials demonstrate just the opposite, showing a neuroprotective effect with aprotinin use (27,113). This research suggests as much as a 60% reduction in the rate of postoperative stroke with the use of aprotinin versus placebo, with one study reporting no incidence of stroke in a high-dose aprotinin group, versus no difference from placebo in the low-dose group (24,27,113,142). In a meta-analysis Sedrakyan et al. (27) concluded that aprotinin use decreased the risk of perioperative stroke, correlating with a 10-event reduction per 1000 patients undergoing CABG and CPB. In contrast, Mangano et al. (38) reported a trend toward increased stroke rates in aprotinin-treated patients, with a significant increase in all cerebrovascular events, as defined by coma, stroke, or encephalopathy. The drawback to the current data is that they are either pooled from various small studies, derived *post hoc*, or come from retrospective analyses. Furthermore, the previous studies had no formal evaluation of stroke (National Institute of Health stroke scale or neurologist examination) or functional recovery (i.e., Barthel, Modified Rankin), which are major limitations. Fortunately, a large, randomized, placebo-controlled trial of aprotinin in cardiac surgery with stroke and cognitive deficits as the primary end-points is in progress.

In the interim, two small prospective studies have been recently published that evaluated the effects of aprotinin on NCD after CPB. Ramlawi et al. (112) reported that the half Hammersmith dose of aprotinin, compared with ϵ-aminocaproic acid, did not have an effect on NCD or markers of brain injury, which included neuron-specific enolase and tau protein. Harmon et al. demonstrated that the full Hammersmith dose of aprotinin decreased the incidence of NCD compared with placebo at 4 days and 6 wk after surgery. Unfortunately, there were no biochemical markers of injury reported (113). Although there is need for further large randomized, controlled trials, these data on NCD, and those cited above concerning

postoperative stroke, would suggest that there is a dose-related effect of aprotinin in decreasing both of these outcomes.

Basic science investigating the effects of aprotinin has shown significant advantages of aprotinin in cerebral protection, and it has revealed some possible mechanisms by which aprotinin may exert it effects and the timecourse in which these effects may be realized. Grocott et al. (114) demonstrated that a single preischemic bolus of aprotinin (30,000 or 60,000 KIU/kg) in rats did not confer neuroprotection in the setting of global or focal cerebral ischemia, as measured by the percentage of dead neurons in the ischemic region, subcortical infarct volume, and cortical infarct volume. Takahashi et al. (115) reported similar results in rats with intracerebroventricular aprotinin dosing (0.3 KIU) during 75 min of complete forebrain ischemia (occlusion of the circle of Willis). However, the same study reported that aprotinin significantly reduced forebrain neuronal cell death and reduced abnormal locomotor activity when given 4 h after the ischemic insult (115). Plasmin and t-PA play an important role in cerebral injury after I/R. Thus, it was hypothesized that, since aprotinin inhibits plasmin but not t-PA (116), plasmin generated by t-PA in the first few hours after forebrain ischemia is essential for delayed neuronal death after transient forebrain ischemia. Furthermore, since plasmin degrades the extracellular matrix, not only directly but also via MMP activation, and since both MMP-2 and MMP-9 play roles in delayed neuronal death (143), their activation by the t-PA/plasmin cascade may be also involved in the delayed neuronal death induced by transient forebrain ischemia. Finally, there is also the possibility that the broad spectrum effects of aprotinin on other serine proteases, such as kallikrein, may be involved in delayed neuronal death (115,144).

Harmon et al. (76) investigated the effects of aprotinin on the interaction of mouse astrocytes exposed to hypoxia-reoxygenation with ICAM-1, an endothelial cell-associated target. Astrocytes exposed to hypoxia produce proinflammatory cytokines and upregulate ICAM-1 on cerebral endothelium. Mouse astrocytes that had been exposed to hypoxia in an anaerobic chamber for 4 h followed by reoxygenation for 24 h were applied to mouse cerebral endothelial cell cultures. The experimental group of endothelial cells was preincubated for 1 h with a very high dose of aprotinin (1600 KIU/mL) before exposure to the astrocytes. ICAM-1 expression was decreased by aprotinin preincubation compared with control (76). It is possible that this reduction of cerebral endothelial ICAM-1 by aprotinin may be a neuroprotective mechanism by which neutrophil transmigration, and thus cerebral I/R, is reduced in patients undergoing CPB.

Finally, two recent studies in large animal models of cardiac surgery with CPB or deep hypothermic circulatory arrest have revealed promising biochemical, histopathological, and functional data concerning the neuroprotective effects of aprotinin (117,118). Durgut et al. (117) investigated the neuroprotective effects of aprotinin (60,000 KIU/kg) and pentoxifylline on S100β protein levels and gliosis in a dog model of CPB (1 h). Pentoxifylline inhibits in vitro activation of neutrophils, including adhesion, chemotaxis, and oxidant release (145). S100β protein is a potential marker for cerebral events during CPB that can indicate injury to the blood–brain barrier or death of glial cells. Increased plasma levels of this protein have been associated with poorer outcomes after postoperative stroke (146,147). Gliosis is an indicator of degeneration and inflammation in the brain (117). There was no difference between preoperative and postoperative S100β protein levels in the aprotinin or pentoxyfilline groups, but there was a significant increase in the placebo group. Furthermore, histopathological examination revealed postoperative gliosis in the placebo and pentoxifylline groups, whereas gliosis was not observed in any of the dogs in the aprotinin group (117).

Anttila et al. also investigated the effects of aprotinin (60,000 KIU/kg) versus control (normal saline) on cerebral injury after CPB and deep hypothermic circulatory arrest in pigs using intravital microscopy. The findings were that, in aprotinin-treated pigs: 1) the mean number of rolling and adherent leukocytes during rewarming was lower than controls, 2) that functional capillary density, a measure of microcirculation flow, recovered faster than controls, and 3) that functional neurologic outcome was significantly improved on postoperative day 1 compared with controls (118). Taken together, these data suggest that aprotinin could attenuate cerebral injury in the setting of CPB through at least three major mechanisms. First, the inhibitory effects of aprotinin on plasmin may reduce extracellular matrix destruction both by directly inhibiting the effects of plasmin on the extracellular matrix and by inhibiting the activation of MMPs by plasmin. Second, the inhibitory effects of aprotinin on CD11b integrin and ICAM-1 reduce neutrophil rolling, adhesion, and extravasation, thereby decreasing proteolytic and oxidative stress on astrocytes and glial cells. Finally, aprotinin has been shown to maintain microvascular flow, possibly alleviating concerns of it being a causal factor in postoperative stroke.

FUTURE DIRECTIONS: CLINICAL AND BASIC RESEARCH

CPB and cardiac surgery induce a coagulopathy, a massive inflammatory response, and I/R injury. Although aprotinin attenuates the coagulopathy, a complete understanding of the relationship between the effects of aprotinin on the inflammatory and I/R cascades and the mechanisms by which aprotinin attenuates organ dysfunction has not been elucidated (2,3).

Aprotinin and the Coagulation Cascade

With respect to the coagulation cascade, numerous questions remain that must be addressed in future research. For instance, there is little data concerning the use of aprotinin in pediatric cardiac surgery. There are also the questions regarding patients with multiple risk factors for bleeding/comorbidity: 1) Which patients should receive full-dose aprotinin? 2) Do baseline coagulation profiles affect the balance of anticoagulant and antithrombotic properties of aprotinin? 3) Because of the great efficacy of aprotinin on postoperative blood product requirements, what are the absolute contraindications to administering aprotinin? Finally, the issue of dosing remains prominent: what is the most effective dose? Is there a toxic limit, and should higher doses be used clinically? An understanding of the efficacy and safety of aprotinin and a reliable pharmacokinetic-based dosing schedule need to be examined in both pediatric and adult patients to find the optimal therapeutic level. The forthcoming prospective, randomized BART Canadian trial should answer some of these questions in the adult population (148). However, some would advocate that trials with doses even higher than the full Hammersmith protocol should be performed (40).

Aprotinin and Inflammation

Many basic science and translational studies are indicating that increasingly higher doses of aprotinin lead to a greater attenuation of the inflammatory response (41). However, because of the interrelatedness of the coagulation, inflammation, and complement systems, this is not an isolated issue. Hence higher dosing regimens should be examined to determine their effects on coagulation and inflammation. Thus, several questions are prominent: 1) Do the effects of aprotinin on the inflammatory system improve hemodynamic outcomes in the cardiac surgical patients? 2) Is there a narrow therapeutic window when this occurs, and is there a dose-dependent effect? 3) Do the same dosing regimens apply for this system as for hemostasis, or would continued dosing into the postoperative period be of benefit due to inflammatory/hemodynamic modulation after I/R?

Aprotinin and Potential Clinical Benefits

With the recent publications by Mangano et al. (38,45) that are contrary to previous studies with respect to mortality and multiorgan morbidity after cardiac surgery with CPB, the future direction of research concerning aprotinin's effects on inflammation and I/R injury in the setting of cardiac surgery must incorporate biochemical and *in vivo* functional analysis of the animal and human heart, lungs, kidney, and brain. This should be done to detail the mechanisms of the novel effect of aprotinin in the preservation of organ function after cardiac surgery and CPB. Thus, the conventional mechanisms of action of aprotinin on the coagulation cascade, its effects

on the inflammatory response, its impact on renal and cognitive function, and its possible novel effects on myocardial dysfunction after I/R in cardiac surgery with CPB all need further clarification with an understanding of the substantial interaction among all of these systems.

In conclusion, past research has shown that aprotinin significantly reduces bleeding during and after cardiac surgery and CPB. We hope to shed light on the current research exploring the role of aprotinin in modulating the effects of cardiac surgery and CPB on inflammation and I/R-mediated organ dysfunction. By understanding these new potential therapeutic targets, and by obtaining a more detailed map of the novel mechanisms of action of aprotinin, its optimal therapeutic window can be established, leading to its efficacious, safe, and economic clinical use in the cardiac surgical patient.

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