J Anesth (2010) 24:96–106
DOI 10.1007/s00540-009-0866-9

REVIEW ARTICLE

Lessons from the aprotinin saga: current perspective on antifibrinolytic therapy in cardiac surgery

Masahiro Ide · Daniel Bolliger · Taro Taketomi · Kenichi A. Tanaka

Received: 7 May 2009 / Accepted: 4 June 2009 / Published online: 29 December 2009

Abstract Antifibrinolytic agents have been prophylactically administered to patients undergoing cardiopulmonary bypass (CPB) to reduce postoperative bleeding due to plasmin-mediated coagulation disturbances. After the recent market withdrawal of aprotinin, a potent bovine-derived plasmin inhibitor, two lysine analogs, ε-aminocaproic acid and tranexamic acid are currently available for clinical use. Although the use of aprotinin recently raised major concerns about postoperative thrombosis and organ dysfunctions, there is a paucity of information on the potential complications related to lysine analogs. Using the available preclinical and clinical data, we present current perspectives on the hemostatic mechanism and potential harms of antifbirnolytic therapy related to cardiac surgery. Fibrin formation is the critical step for hemostasis at the site of vascular injury, and localized fibrinolytic activity counterbalances excess fibrin formation which might result in vascular occlusion. Inhibition of the endogenous fibrinolytic system may be associated with thrombotic complications in susceptible organs. It is thus important to understand CPB-related changes in endogenous fibrinolytic proteins (e.g., tissue plasminogen activator (tPA), plasminogen) and antifibrinolytic proteins (e.g., α_2-antiplasmin).

M. Ide
Kobe Anesthesia Associates, Hyogo, Japan

D. Bolliger · T. Taketomi · K. A. Tanaka (✉)
Department of Anesthesiology, Emory University School of Medicine, 1364 Clifton Road, NE, Atlanta, GA 30322, USA
e-mail: ktanaka@emory.edu

Keywords Antifibrinolytic therapy · Complications · Aprotinin · ε-Aminocaproic acid · Tranexamic acid

Introduction

Perioperative bleeding is a serious complication that adversely affects the morbidity and mortality of cardiac surgery [1–3]. Coagulation abnormalities after cardiopulmonary bypass (CPB) consist of multiple alterations including a consumptive or dilutional loss of coagulation factors and platelets, activation of fibrinolysis, and various metabolic derangements, for example hypothermia and acidosis [4]. Antifibrinolytic agents are prophylactically administered during CPB to improve the stability of fibrin clots against plasmin-mediated degradation (Fig. 1) [5]. Two antifibrinolytic agents, ε-aminocaproic acid and tranexamic acid, were originally developed by Okamoto et al. [6] based on the structure of lysine (hence lysine analogs). Aprotinin, a bovine-derived natural protease inhibitor, has been used as a plasmin inhibitor, but has recently been withdrawn from the market after higher 30-day morbidity and mortality with aprotinin compared with lysine analogs were reported in a prospective Canadian antifibrinolytic trial (blood conservation using anti-fibrinolytics in a randomized trial (BART)) [7]. Aprotinin has long been regarded as a more potent antifibrinolytic and anti-inflammatory than lysine analogs, because aprotinin directly inhibits multiple serine proteases that are activated in the CPB circuit (Table 1) [8]. Lysine analogs reduce activation of plasminogen to plasmin by occupying the lysine binding site of plasminogen, but they do not directly antagonize enzymatic actions of plasmin [5, 6]. Could it be possible that pharmacological differences between aprotinin and lysine analogs lead to different clinical effects and

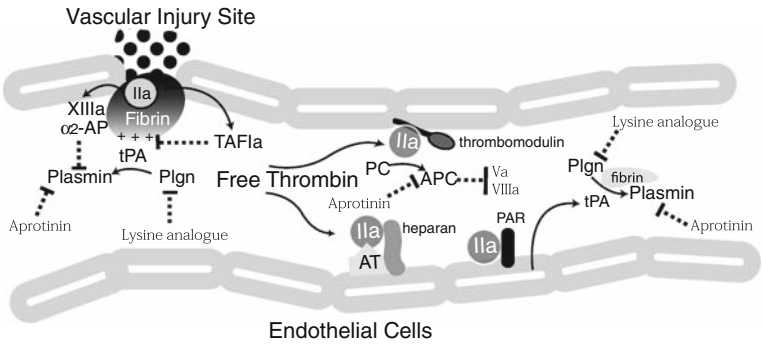

Fig. 1 Local and systemic regulation of coagulation and fibrinolysis. Hemostasis is established at the vascular injury site after fibrin is polymerized by thrombin (*IIa*) and activated factor XIII (*XIIIa*). Factor XIIIa and thrombin-activatable fibrinolysis inhibitor (*TAFI*) are both activated by thrombin, and they play important roles in stabilizing fibrin against plasmin. Aprotinin and lysine analogs inhibit fibrinolysis by different mechanisms. When thrombin is released (i.e., free thrombin) into systemic circulation during hemostatic activation, antithrombin (*AT*) and thrombomodulin of intact endothelium bind to thrombin, and reduce its procoagulant activity. Thrombomodulin-bound thrombin activates protein C (*APC*), which inactivates coagulation factors Va and VIIIa. Thrombin also causes the release of tissue plasminogen activator (*tPA*) from endothelium, which promotes plasminogen (*Plgn*) conversion to plasmin on the fibrin surface. *Broken lines* indicated inhibitory action of respective protease inhibitors. α_2-*AT*, α_2-antiplasmin; *PAR*, protease-activated receptor (thrombin receptor on endothelium)

Table 1 Inhibitory effects of aprotinin, epsilon-aminocaproic acid, and tranexamic acid

	Aprotinin	EACA	Tranexamic acid
Molecular weight	6,512	131	157
Plasma level [mg/dL][a]	4.2	60	3.3
[Mol.]	6.4×10^{-6}	4.6×10^{-3}	2.1×10^{-4}
K_i values [Mol.]			
Plasmin	7×10^{-11}	3.2×10^{-1}	1.6×10^{-2}
Kallikrein	3.6×10^{-8}	NS	NS
Thrombin	6.1×10^{-5}	NS	NS
FXIa	1.1×10^{-6}	NS	NS
APC	1.1×10^{-6}	NS	NS

The lower the K_i value, the more avidly target enzyme activity is inhibited by the respective antifibrinolytic agent [8]

Mol. mol/L, *EACA* ε-aminocaproic acid, *FXIa* activate factor XI, *APC* activated protein C, *NS* no significant inhibition

[a] Peak levels after a bolus intravenous dose of the respective antifibrinolytic agent (data from Refs. [9–11])

outcomes in hemostasis and major organ function in cardiac surgery [7, 12, 13]?

In this review, both preclinical and clinical data on coagulation related to the fibrinolytic system will be discussed to provide insights into the clinical usefulness and potential harms of antifibrinolytic therapy.

Endogenous regulation of fibrinolysis

The fibrinolytic response is normally a localized reaction that depends on the presence of fibrin (Fig. 1) [14]. Two serine proteases, tissue plasminogen activator (tPA) and plasmin, are rapidly inhibited in the plasma phase by the serine protease inhibitors plasminogen activator inhibitor-1 (PAI-1) and α_2-antiplasmin, respectively (Table 2) [15]. However, tPA and plasminogen preferentially bind to positively charged lysine residues expressed on fibrin, and this co-localization of tPA and plasminogen increases the efficiency of plasmin activation and subsequent fibrin degradation. Although fibrinolysis is a normal physiologic response, to dissolve excess fibrin formed within blood vessels, premature breakdown of fibrin may increase re-bleeding. There are several mechanisms which stabilize the fibrin (clot) against fibrinolytic enzymes at the site of vascular injury. Platelets contain PAI-1 in the α-granule [16], and locally released PAI-1 upon platelet activation renders platelet-rich thrombin resistant to fibrinolysis [17]. The zymogen factor (f)XIII is activated by thrombin to transglutaminase fXIIIa, which cross-links α_2-antiplasmin to fibrin α chains [18]. This reaction proceeds more rapidly than fXIIIa-mediated fibrin cross-linking, and therefore fibrin polymers become resistant to fibrinolysis in the early phase of blood coagulation. Congenital α_2-antiplasmin deficiency is a rare disorder, but affected patients develop severe bleeding tendency because of increased susceptibility to fibrinolysis [19]. Thrombin is also involved in activation of a pro-carboxypeptidase, thrombin activatable fibrinolysis inhibitor (TAFI). Later in the course of coagulation a high local concentration of thrombin (>150 nM) is achieved to form activated TAFI (TAFIa) inside the clot. TAFIa cleaves carboxyterminal lysine residues from the fibrin, thereby preventing the binding of plasminogen [20, 21]. The antifibrinolytic effect of TAFIa is localized to the site of injury, because of the need for the high thrombin concentration and the short half-life of TAFIa (8–15 min)

Table 2 Endogenous factors involved in the regulation of fibrinolysis

	Molecular weight (Da)	Concentration (mg/dL)	Function
Fibrinolysis promotor			
tPA	68,000	0.0005	Activate plasminogen to plasmin
uPA	54,000	0.0002	Activate plasminogen to plasmin
Plasminogen	92,000	20	Zymogen of plasmin
Prekallikrein	88,000	4	Activate scuPA to uPA as kallikrein
Factor XII	80,000	3	Activate prekallikrein to kallikrein as factor XIIa
Fibrinolysis inhibitor			
PAI-1	52,000	0.001	Inhibitor of tPA and uPA
α_2-Antiplasmin	70,000	7	Inhibitor of plasmin
Pro-TAFI	60,000	0.5	Reduce plasminogen binding to fibrin as TAFI
Factor XIII	320,000	3	Cross-link α_2-antiplasmin to fibrin
C_1-inhibitor	105,000	18	Inhibitor of kallikrein, factors XIa and XIIa

tPA tissue plasminogen activator, *uPA* urokinase-type plasminogen activator, *scuPA* single chain uPA, *TAFI* thrombin activatable fibrinolysis inhibitor

[22]. Taken together, endogenous antifibrinolytics, PAI-1, α_2-antiplasmin, and TAFIa, are highly concentrated at the focal point of blood coagulation according to the gradient of activated platelets, fXIIIa, and thrombin [18, 20]. Thus, fibrin near the vessel wall is highly resistant to fibrinolysis whereas intraluminal fibrin is more accessible by fibrinolytic enzymes for re-canalization of the injured blood vessel [23].

Cardiopulmonary bypass and fibrinolysis

The rationale for administering antifibrinolytic agents during CPB is to reduce plasmin activation and plasmin-mediated hemostatic perturbations (Fig. 1). Several mechanisms are attributed to the up-regulation of fibrinolytic activity during CPB. The release of tPA from Weibel–Palade bodies (WPB) of endothelium is stimulated by thrombin [24], epinephrine [25], vasopressin, desmopressin [26], bradykinin and other substances [27]. Several investigators demonstrated that the peak tPA level occurs early (30–60 min) during CPB without antifibrinolytic therapy [28–30]. Interestingly, factor VIII and von Willebrand factor (vWF) are also released from WPB. Factor VIII and vWF level are minimally affected by the initiation of CPB whereas other factors (prothrombin, factors V, VII, IX, and X) are reduced to 28–60% of baseline [31, 32]. Taken together, the initiation of CPB seems to dynamically stimulate WPB granule release from endothelium by humoral or rheological mechanisms [33]. Does systemic fibrinolysis result from a rapid surge of tPA at the beginning of CPB? The answer is "highly unlikely" for two reasons. First, PAI-1 rapidly binds to tPA, and plasma PAI-1 level is at its lowest level after 30 min of CPB [29, 30]. Second, fibrin that catalyzes the interaction of tPA and plasminogen is minimal in the start of CPB. Over the course of CPB, plasmin generation measured as plasmin-α_2-antiplasmin (PAP) complex is progressively increased (without antifibrinolytics) in response to fibrin formation, due to incomplete suppression of thrombin by heparin-AT complex during CPB (Fig. 2) [28, 30]. Postoperatively, there is a gradual increase of PAI-1 secretion (its peak at 2–4 h after CPB) that suppresses fibrinolytic responses [29, 30]. These CPB-related dynamic changes in endogenous elements/regulators of fibrinolytic system are summarized in Fig. 3.

In addition to cleaving fibrin into fibrin degradation products, plasmin is known to degrade coagulation factors such as fV and fVIII [34, 35]. Plasmin may also affect platelet function by modulating the surface glycoprotein (GP) Ib or GPIIb/IIIa receptors [36], and protease-activated receptor-4 (PAR-4) [37]. The prophylactic use of antifibrinolytic agents has been widely implemented after several pivotal trials in cardiac surgery demonstrated its blood-sparing effects [38, 39]. Either aprotinin or tranexamic acid reduces plasma markers of fibrin degradation (D-dimer), although aprotinin seems to preserve endogenous α_2-antiplasmin by directly inhibiting plasmin [30, 40]. A number of meta-analyses demonstrated that aprotinin and lysine analogs reduce post-operative erythrocyte transfusion, and the risk of re-exploration, compared with the placebo [41, 42]. Also, in a prospective randomized study of patients who recently received clopidogrel (within 5 days of surgery), aprotinin was shown to reduce postoperative bleeding compared with placebo (760 ± 350 vs. $1{,}200 \pm 570$ mL, $p < 0.001$), and the transfusion of erythrocytes and platelets after coronary bypass graft surgery (mostly performed under CPB) [43].

As mentioned previously, persistent platelet activation, and fibrinogen conversion to fibrin monomers, occur when

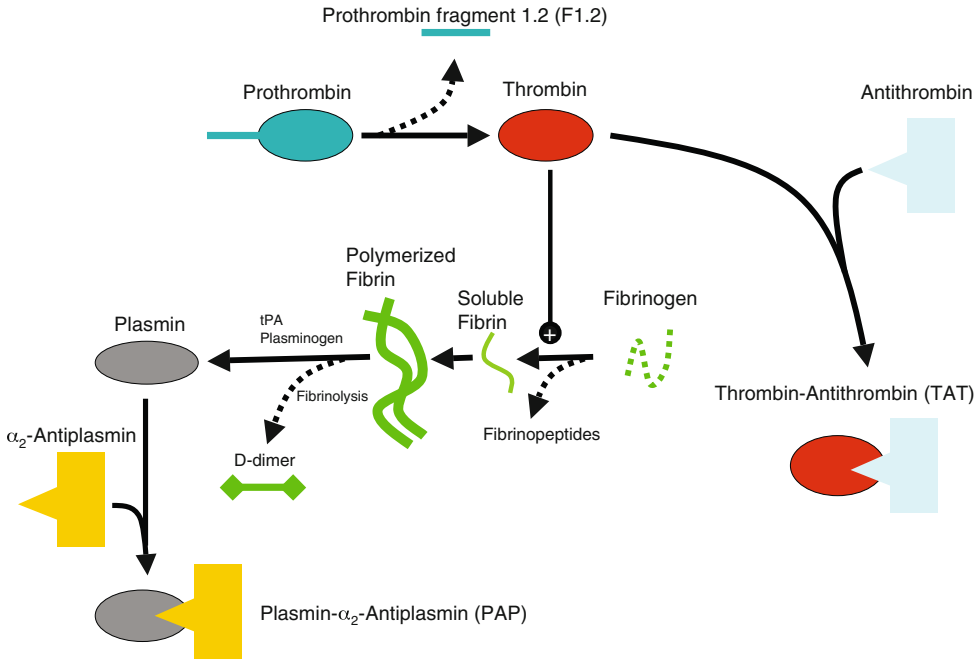

Fig. 2 Plasma markers of coagulation and fibrinolysis. Prothrombin fragment 1.2 is released in the conversion of prothrombin to thrombin. Thrombin–antithrombin complex (*TAT*) is increased in the procoagulant state. Thrombin mediates the conversion of fibrinogen to soluble fibrin (monomer), which is polymerized by activated factor XIII to fibrin at the site of vascular injury (Fig. 1). Fibrinolytic response is triggered on fibrin which adsorbs tissue plasminogen activator (*tPA*) and plasminogen to form plasmin. D-dimer is released after plasmin-mediated degradation of fibrin. Plasmin is inhibited by α_2-antiplasmin

thrombin inhibition is insufficient because of heparin or antithrombin (AT) deficiency [44, 45]. Heparin is a mixture of high and low-molecular-weight glycosaminoglycans (3,000–30,000 Da), and it catalyzes AT-mediated inhibition of thrombin and activated factor X (fXa) [46]. Low AT activity (50–60% of normal) is observed in 10–20% of patients receiving preoperative heparin therapy, because heparin mediates rapid AT turnover [47, 48]. Preexisting procoagulant stimuli (e.g., sepsis, acute heparin-induced thrombocytopenia [49]) can exacerbate deficiency of AT and other endogenous anticoagulants (e.g., proteins C and S). Plasmin formation triggered by intravascular fibrin (secondary fibrinolysis) plays an important role in preventing fibrin deposition, and maintaining blood flow in vital organs [27]. Thus antifibrinolytic regimens are potentially harmful in patients with ongoing intravascular coagulation and fibrinolysis. Preexisting intravascular coagulation and secondary fibrinolysis can be estimated by measuring fibrin monomer (soluble fibrin) [50], D-dimer [51], and PAP complex [52] (Figs. 2, 3). However, these tests are not routinely used in the preoperative evaluation because standardized cut-off values do not exist, and sensitivity and specificity vary widely among different assay techniques [53].

Is antifibrinolytic therapy associated with organ dysfunction?

Patients with cardiovascular disease often present with elevated acute inflammatory markers, for example C-reactive protein (CRP), fibrinogen, and PAI-1 [54]. Thrombolytic therapy using tPA or urokinase-type plasminogen activator may be used in the treatment of acute coronary artery thrombosis or intracerebral thrombosis. The safety of intraoperative antifibrinolytic therapy has thus been a matter of debate for some time [55]. Although the analysis of the graft patency in prospective randomized trials of aprotinin (aprotinin, $n = 436$ vs. placebo, $n = 434$) revealed no increased venous graft occlusion [56], Mangano et al. [57] expressed strong concerns about organ dysfunction after analyzing a large database of coronary bypass patients ($n = 5{,}022$) who received aprotinin or lysine analogs on CPB. In this retrospective study, they observed reduced mortality (1.3 vs. 4.0%, $p < 0.001$) and nearly 50% reduction in the rate of myocardial infarction and stroke among those who received aspirin (up to 650 mg) within 48 h of surgery. They also noticed higher mortality with antifibrinolytic therapy among patients who received aspirin in the early postoperative period (Fig. 3B of Mangano et al. [57]). On further analysis of the same database using a multivariate logistic regression and propensity-score adjustments, Mangano et al. [12] demonstrated that renal dysfunction and dialysis were 8% in the aprotinin group ($n = 1{,}295$) compared with 3% in the control (no antifibrinolytics, $n = 1{,}374$). Also observed were increased tendencies for renal dysfunction, acute myocardial infarction, heart failure, peripheral vascular occlusion, cerebral thromboembolism with aprotinin, but neither ε-aminocaproic acid ($n = 883$) nor tranexamic acid ($n = 822$) was found to be associated with such events. In the follow-up study of these patients groups, 5-year mortality was found to be higher with aprotinin (20.8%) than with controls (12.7%), ε-aminocaproic acid (15.8%), and

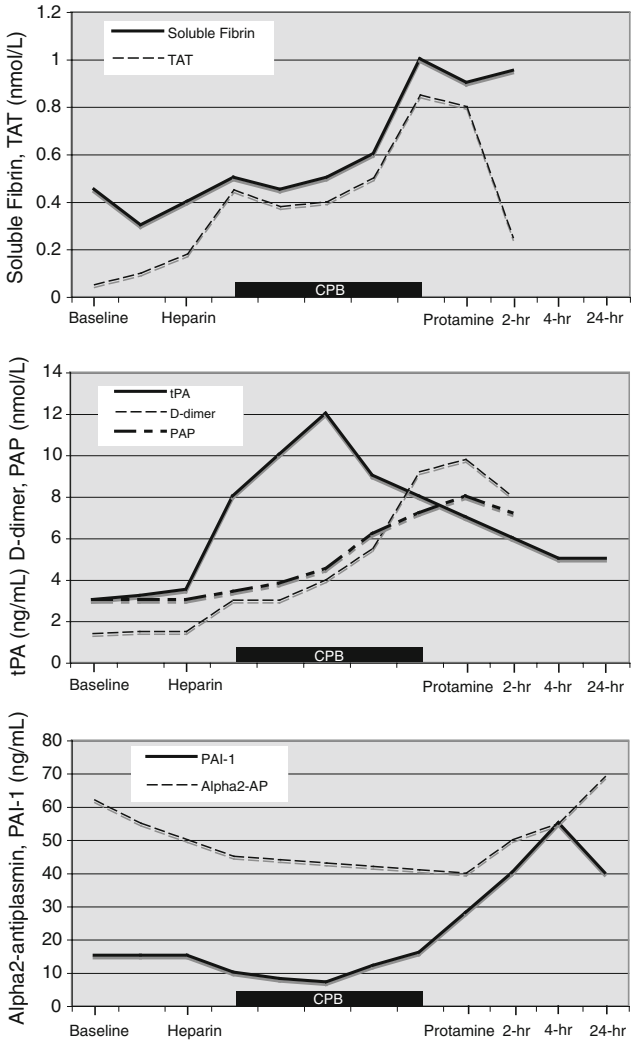

Fig. 3 Changes in coagulation markers and fibrinolytic activity during CPB. *Top panel* Plasma levels of soluble fibrin and thrombin-antithrombin complex (*TAT*) are progressively increased, reflecting persistent thrombin generation during CPB. *Middle panel* The peak plasma level of tPA is observed early during CPB, whereas plasmin generation (plasmin–α_2-antiplasmin complex; *PAP*) and fibrinolysis (*D-dimer*) markers are increased toward the end of CPB similar to soluble fibrin and TAT levels. *Lower panel* The lowest level of plasminogen activator-1 (*PAI-1*) coincides with the peak level of tPA. Plasma α_2-antiplasmin is more gradually decreased during CPB

tranexamic acid (14.7%) [13]. However, is this sufficient evidence to conclude aprotinin is more harmful than lysine analogs? In a retrospective study, statistical adjustment is generally incomplete in eliminating unmeasured or unidentified factors (i.e., confounding variable) which may spuriously affect a causal relationship. For example, aprotinin is more likely to be used in high-risk patients if the use of antifibrinolytic therapy is not randomized. In fact, 42.8% of patients in the aprotinin group had preoperative heart failure (vs. 27–33% in lysine analog groups) [12, 13]. It is speculated that 5-year post-operative morality

Table 3 Comparison of the Mangano and BART studies

Mangano et al.	BART
Type of study	
Retrospective	Prospective randomized
Study sites	
69 sites worldwide	19 sites in Canada
Periods	
Nov 1996–Jun 2000	Aug 2002–Oct 2007
Sample size	
5,022	2,311
Type of surgery	
CABG	CABG + valve
CABG + valve	MVP/MVR
	Asc Ao/Arch Repl.
Findings related to aprotinin	
↑Renal dysfunction	↑Myocardial infarction
↑Mortality	↑Right heart failure
	↑Hemorrhagic death

Data summarized from Refs. [7, 12, 13]

CABG coronary bypass grafting surgery with CPB, *valve* valve-replacement surgery with CPB, *MVP* mitral valvuloplasty, *MVR* mitral valve replacement, *Asc Ao Repl.* ascending aortic replacement, *Arch Repl.* aortic arch replacement

rate would be higher in severe patients in whom aprotinin was frequently indicated. A prospective randomized trial was necessary to resolve this issue, and the BART study was initiated to compare aprotinin, ε-aminocaproic acid, and tranexamic acid in high-risk cardiac surgery. This study was prematurely stopped when 2,311 patients were enrolled because the 30-day post-operative mortality rate was higher in the aprotinin group than in the others (this study was planned to enroll 2,970 patients) [7]. Consequently, aprotinin was voluntarily taken off the market in November 2007. However, there are a number of limitations to conclusively support of Mangano's data by the BART results (Table 3). In Mangano's study, patients underwent primary coronary artery bypass surgery, whereas in the BART study, patients had more complex surgery (CABG with valve replacement, multiple valve replacements, replacement of ascending aorta or aortic arch). Furthermore, the results could be influenced by differences among institutional practices, surgical skills, and patients' characteristics, because these studies were conducted as a multicenter study in a different time period (Table 3). In contrast with Mangano's studies, there were no overall differences in the rates of renal dysfunction/hemodialysis, stroke, cardiac events, or cardiogenic shock in the BART study [7, 12]. In addition, a trend of lower incidence of massive hemorrhage was observed with aprotinin (9.5 vs. 12.5% in groups receiving lysine analogs). Nevertheless, for 108 patients who died within

30 days of surgery, the incidence of myocardial infarction, cardiogenic shock, right heart failure, and the risk of massive blood loss were higher with aprotinin than with lysine analogs. Some information that may directly or indirectly affect outcomes cannot be obtained from these studies. For example, the duration of CPB and the amount of heparin are not summarized, although Mangano et al. had previously demonstrated various predictors for post-operative renal dysfunction including catecholamine uses, intra-arterial balloon pump, and prolonged CPB lasting over 2 h (antifibrinolytic therapy was not regarded as a risk factor) [58]. The series of studies on aprotinin and lysine analogs in cardiac surgery obviously demonstrate that blood-sparing effects of antifibrinolytic therapy is not risk-free, and it may even worsen prognosis in certain high-risk cardiac surgical patients.

Does blockade of the endogenous fibrinolytic system increase thrombosis?

There is a paucity of clinical data on hypercoagulability and secondary fibrinolysis in cardiac surgery. Therefore, a review of preclinical studies may aid understanding of procoagulant aspects of antifibrinolytic therapy. A number of animal studies have previously demonstrated that fibrinolysis plays important but variable roles in maintaining the function of different organs. The fibrinolytic system functions in an organ-specific manner, and the effect of antifibrinolytic therapy is different in various organs [59, 60]. For example, uPA is highly expressed in the murine kidney, and glomerular fibrin deposition was increased after exposure to gram-negative bacterial endotoxin, because the latter down-regulates uPA expression [59]. Fibrin deposition in the lung, heart, and hepatic sinusoids was much less than in kidneys after exposure to the endotoxin, but they were markedly increased when ε-aminocaproic acid was co-administered with the endotoxin. In double-knockout mice of uPA and tPA, the incidence of growth retardation, poor wound healing, and death were higher than for single knockout of uPA or tPA [61]. The degree of fibrin deposition in the lung, liver, intestine, and reproductive system were significantly increased relative to the single knockouts. Although tPA-deficient mice have severely reduced ability to break down preformed fibrin clots, they were less susceptible to spontaneous venous fibrin deposition than uPA-deficient or uPA/tPA-deficient mice.

The interaction of coagulation and fibrinolysis was elegantly demonstrated in a study of rabbits with elevated plasma PAI-1 after exposure to endotoxin or recombinant PAI-1 [62]. When plasma tPA and uPA were inhibited by excess PAI-1, fibrin deposition was increased in the lung, liver, kidney, and spleen after injection of ancrod (the snake venom extract which forms intravascular fibrin without thrombin). Interestingly, there were no increases in fibrin deposition except for small glomerular fibrin deposits when thrombin was infused in rabbits with elevated plasma PAI-1. This striking difference between ancrod and thrombin can be explained by the presence of endogenous inhibitors for thrombin activity. Intravenously injected thrombin is rapidly suppressed by AT and endothelium-bound thrombomodulin [63], whereas ancrod is not neutralized by protease inhibitors in plasma. In another study, preformed fibrin monomer was infused in rabbits undergoing heparin anticoagulation [64]. In animals pretreated with ε-aminocaproic acid, fibrin deposits were significantly increased in glomeruli, but not in the lung or liver. Taken together, these data support the concept that various organs are differentially regulated in terms of coagulation and fibrinolysis (e.g., expression of tissue factor, thrombomodulin, uPA and tPA) under normal and disease states, and thus some organs are more susceptible to fibrin depositions than others [59, 60, 65, 66].

The systemic impact of thrombin generation has been evaluated in several non-human primate models [67–69]. Giles and Taylor elegantly demonstrated endothelium-mediated anticoagulation and fibrinolytic functions which neutralize excessive intravascular formation of thrombin and fibrin after intravenous infusion of fXa and phospholipids in baboons [67, 68]. Intravascular thrombin formation elicits activation of two serine proteases, activated protein C (APC) and plasmin. After binding of thrombin (via exosite I) to endothelial thrombomodulin, the catalytic activity of thrombin is optimized toward protein C and TAFI [70]. APC inhibits fVa, fVIIIa, and PAI-1, thus preventing continuous thrombin generation, and promotes fibrinolysis [70–72]. Thrombin stimulates a rapid and transient release of tPA from WPB of endothelium, promoting plasmin activation [24, 27]. Increased systemic levels of APC and D-dimers after fXa/phospholipid or thrombin infusion reflect potent anticoagulant mechanisms to prevent systemic thrombosis [67–69]. What would happen if antithrombotic protease activities were inhibited? When APC was neutralized by use of a specific monoclonal antibody in baboons, tPA and D-dimer levels were significantly increased whereas plasma levels of fV, fVIII, α_2-antiplasmin, and fibrinogen were reduced [68]. Thus APC and plasmin have complementary roles in the suppression of thrombin activity and dissolution of intravascular fibrin (Fig. 1).

These preclinical data may not seem to have any implication in cardiac surgery when heparin anticoagulation is routinely used to suppress thrombin activity. However, heparin anticoagulation may inadvertently fail in some cases because of inadequate heparin dosing or low

AT activity [44, 73]. The inflammatory state during CPB results in activation of neutrophils and monocytes, further reducing endogenous anticoagulant activity by releasing elastase [74], expressing tissue factor [75], or modulation of endothelial cells (e.g., reduced thrombomodulin expression) [76, 77]. In patients who develop severe postoperative hemorrhage after CPB, not only procoagulant elements but also anticoagulant elements are deficient [73, 78]. Without adequate control of thrombin activity, the risk of intravascular coagulation and the hazard of antifibrinolytics would be enhanced (Fig. 1). Bleeding patients are more likely to receive procoagulant interventions to assist hemostasis in addition to antifibrinolytic therapy. Rapid, uncontrolled systemic thrombus formation has been reported in a number of cases [73]. Although antifibrinolytic therapies are often implicated as a cause of such thromboses, it is prudent to pay attention to the regulation of thrombin, because fibrin (clot) formation always precedes protein C activation and plasmin activation [79, 80]. It is thus important to recognize the risk of systemic thrombin activation after other hemostatic interventions, for example platelet concentrates [81], recombinant fVIIa [82], or prothrombin complex concentrates [83], when endogenous anticoagulant mechanisms are dysfunctional [84, 85].

Are lysine analogs safer than aprotinin?

Epsilon-aminocaproic acid and tranexamic acid have much smaller molecular weights (131 and 157 Da, respectively) than aprotinin (6,512 Da). Lysine analogs are far less antigenic than aprotinin which is associated with allergic/anaphylactic reactions upon repeat exposure [86]. Lysine analogs are available in oral and intravenous formulations, because they are indicated in hemophiliac patients (e.g., dental extraction). Although thrombotic complications are rare in hemophilia, several cases of glomerular capillary thrombosis after ε-aminocaproic acid had been reported [87, 88]. Tranexamic acid has been associated with seizure episodes in several case reports and a retrospective cardiac surgery study [89, 90]. Because lysine analogs cross the blood–brain barrier to enter central nervous system, potential hyperexcitability of neurons should be cautioned [91] when tranexamic acid is being used at a higher dose (>40 mg/kg iv per day). Martin et al. [90] recently presented follow-up data from 1,188 consecutive patients who received aprotinin in the first 5 months ($n = 596$), and tranexamic acid in the following 5 months at three centers (September 2005–June 2006). Aprotinin was administered as a 2×10^6 KIU bolus intravenously and in the CPB prime, followed by continuous infusion of 0.5×10^6 KIU/h until chest closure. Tranexamic acid was administered as a 2 g bolus intravenously and in the CPB prime, followed by continuous infusion of 0.5 g/h until chest closure. They analyzed the 1-year outcome in relation to the antifibrinolytic therapy, and included post-hoc analysis according to the type of surgery—primary coronary bypass graft surgery (CABG), primary valve surgery, and high-risk surgery (combined CABG/valve, redo, and aortic surgery). There was a significant reduction in chest tube drainage up to 24 h with aprotinin compared with tranexamic acid, although only a small difference was observed in the postoperative use of red blood cells and fresh frozen plasma. Notably, the incidence of seizure was much higher with tranexamic acid than with aprotinin (4.6 vs. 1.2% in all patients, $P < 0.001$). In valve surgery patients, persistent atrial fibrillation and renal failure were also more prevalent with tranexamic acid than with aprotinin. Relative to tranexamic acid, aprotinin was associated with a higher incidence of acute myocardial infarction (5.8 vs. 2.0%, $P < 0.027$) and renal dysfunction (serum creatinine >1.3 mg/dL, an increase ≥0.5 mg/dL over the baseline) (22.5 vs. 15.2%, $P < 0.036$). One-year mortality was also higher after aprotinin than tranexamic acid in the high-risk surgery group (17.7 vs. 9.8%, $P < 0.034$). Their findings partially support the concerns about aprotinin-associated organ dysfunction which were raised by Mangano et al. and the BART investigators.

According to the approved labels, aprotinin is indicated for CABG with CPB, and tranexamic acid is indicated for patients with hemophilia undergoing dental extraction. It is not uncommon to prescribe a medication "off-label" to a population of patients that is excluded or untested in the clinical trials that led to the original approval. For example, 23% of patients currently treated with vitamin K antagonist (e.g., warfarin) for prevention of thromboembolism do not meet the eligibility criteria of major clinical trials that demonstrated vitamin K antagonist therapy as safe and efficacious [92]. Forty percent of those who were admitted with vitamin K antagonist-induced bleeding had one or more exclusion criteria. As the number of exclusion criteria was increased, the risk of bleeding was multiplied threefold for one criterion and up to 15-fold for more than 2 criteria. Analogously, series of antifibrinolytic trials related to aprotinin in a variety of cardiac surgery patients demonstrated that antifibrinolytic therapy caused unforeseeable adverse events which were not evident in the original phase III trials. As suggested by Martin et al., the overall incidence of complications seems to depend on the target population [90]. Potent blockade of fibrinolytic pathways with aprotinin may adversely increase the incidence of acute myocardial infarction in CABG patients, whereas use of tranexamic acid in valve surgery is associated with postoperative seizures. The benefit of antifibrinolytic therapy in patients undergoing noncomplex cardiac surgery

Table 4 List of factor concentrates

Concentrate	Indication	Product	Manufacturer	Viral inactivation
Fibrinogen	Fibrinogen def	Clottagen	LFB	TNBP/polysorbate 80
		Riastap	CSL Behring	Pasteurization, 60°C 20-h
FXIII	FXIII def	Fibrogammin	CSL Behring	Pasteurization, 60°C 10-h
		rFXIII	ZymoGenetics	Not indicated
rFVIIa	Hemophilia, FVII def	Novoseven	NovoNordisk	Not indicated
aPCC	Hemophilia	FEIBA	Baxter	Vapor heated, 60°C 10-h then 80°C 1-h
PCC	VKA reversal	Octaplex	Octapharma	TNBP/polysorbate 80, nanofilteration
		Beriplex	CSL Behring	Pasteurization, 60°C 10-h, nanofilteration

FXIII factor XIII, *rFXIII* recombinant factor XIII, *TNBP* tri-[*n*-butyl]-phosphate, an organic solvent which removes lipids (c.f., [100]), *rFVIIa* recombinant activated factor VII, *aPCC* activated prothrombin complex concentrate, *PCC* prothrombin complex concentrate, *VKA* vitamin K antagonist (full PCC products which include factors II, VII, IX, X, protein C and S are listed, c.f. Ref. [101])

(e.g., off-pump coronary bypass) is relatively small because the extent of hemodilution is not significant enough to depress endogenous antifibrinolytic proteins (e.g., α_2-antiplasmin) and enhance fibrinolytic pathways [93, 94]. The indication of antifibrinolytic therapy may be individually evaluated using a point-of-care coagulation monitor, for example thromboelastometry and Sonoclot [94, 95].

Future perspectives and conclusion

In the course of blood coagulation, activation of fibrinolytic enzymes is the late event which balances hemostasis and vascular patency [96, 97]. Fibrin formation is a triggering mechanism for fibrinolytic activation, because adsorption of tPA and plasminogen by fibrin enables efficient plasmin activation [97]. Antifibrinolytic agents prevent premature breakdown of the fibrin clot at the vascular injury site. The maintenance of plasma fibrinogen concentration is thus important for antifibrinolytic therapy to be efficacious [94]. Indeed, a low fibrinogen level is an important predictor of post-CPB bleeding [98, 99]. Purified fibrinogen concentrates from human plasma have been used in the management of congenital afibrinogenemia and acquired bleeding tendency in Europe and other countries (Table 4). It is extremely difficult to raise plasma fibrinogen and other factors using fresh frozen plasma alone (e.g., 20 U FFP would be necessary to increase fibrinogen by 100 mg/dL) [102]. Therefore, purified fibrinogen and other coagulation factor concentrates with virus inactivation (Table 4) are favorable alternatives to cryoprecipitate and fresh frozen plasma to reduce transfusion-related complications [103].

The "off-label" use of recombinant activated factor VII (rFVIIa; Novoseven, Bagsbaerd, Denmark) was originally described as a rescue intervention in post-CPB bleeding cases, but there have been several randomized cardiac surgical studies [104, 105]. Although intravascular thrombosis after rFVIIa is rare even at high doses (200–300 μg/kg) in hemophilia patients with inhibitors, the incidence of thrombosis was found to be higher in non-hemophiliac patients, particularly after surgery [106]. Because AT and other endogenous anticoagulant levels are reduced in cases requiring prolonged CPB [73], it is prudent to administer a smaller dose (20–45 μg/kg) than the standard dose (90–120 μg/kg for hemophilia) [105].

As a potential alternative to aprotinin, Dietrich et al. reported a novel, synthetic, small protease inhibitor called CU-2010 (molecular weight, 700 Da) [107]. Its high affinity for plasmin (K_i, 2 nM) is comparable with that of aprotinin (K_i for plasmin, 4 nM), and it is approximately tenfold more potent than tranexamic acid. CU-2010 is presumably less antigenic than aprotinin, because of its small size, but an additional clinical study is necessary to establish its indication, efficacy, and safety.

In conclusion, antifibrinolytic therapy has become the mainstay hemostatic strategy for most cardiac surgery procedures using CPB. However, we have learnt from the aprotinin sage that the routine use of antifibrinolytics in a diverse cardiac surgical population may be associated with various adverse events which had not been evident from previous proof-of-efficacy trials. Understanding in-vivo regulatory mechanisms and pharmacologic modulation of fibrinolysis is important, and additional laboratory and clinical studies are necessary to determine the optimum indication and safe antifibrinolytic regimens. With regard to antifibrinolytic therapy, we can still learn from the cautionary words of Dr Oscar Ratnoff [87], in 1969, on current practice; "Epsilon-aminocaproic acid is a useful weapon. As with all potentially lethal weapons, the key to use is circumspection."

Acknowledgments This work was supported in part by the Department of Anesthesiology, EmoryUniversity School of Medicine

Conflict of interest statement Dr. Tanaka has previously received the research support from the Bayer Healthcare.

References

1. Dacey LJ, Munoz JJ, Baribeau YR, Johnson ER, Lahey S, Leavitt BJ, et al. Reexploration for hemorrhage following coronary artery bypass grafting: incidence and risk factors. Arch Surg. 1998;133:442–7.
2. Koch CG, Li L, Duncan AI, Mihaljevic T, Loop FD, Starr NJ, et al. Transfusion in coronary artery bypass grafting is associated with reduced long-term survival. Ann Thorac Surg. 2006;81:1650–7.
3. Koch CG, Li L, Sessler DI, Figueroa P, Hoeltge GA, Mihaljevic T, et al. Duration of red-cell storage and complications after cardiac surgery. N Engl J Med. 2008;358:1229–39.
4. Despotis GJ, Gravlee G, Filos K, Levy J. Anticoagulation monitoring during cardiac surgery: a review of current and emerging techniques. Anesthesiology. 1999;91:1122–51.
5. Mannucci PM, Levi M. Prevention and treatment of major blood loss. N Engl J Med. 2007;356:2301–11.
6. Okamoto S. Strategies for creating new medicines. Kobe: Kobe Research Project on Thrombosis and Hemostasis; 2003.
7. Fergusson DA, Hebert PC, Mazer CD, Fremes S, MacAdams C, Murkin JM, et al. A comparison of aprotinin and lysine analogues in high-risk cardiac surgery. N Engl J Med. 2008;358:2319–31.
8. Stassen JM, Lambeir AM, Matthyssens G, Ripka WC, Nystrom A, Sixma JJ, et al. Characterisation of a novel series of aprotinin-derived anticoagulants. I. In vitro and pharmacological properties. Thromb Haemost. 1995;74:646–54.
9. Royston D, Cardigan R, Gippner-Steppert C, Jochum M. Is perioperative plasma aprotinin concentration more predictable and constant after a weight-related dose regimen? Anesth Analg. 2001;92:830–6.
10. Bennett-Guerrero E, Sorohan JG, Canada AT, Ayuso L, Newman MF, Reves JG, et al. Epsilon-aminocaproic acid plasma levels during cardiopulmonary bypass. Anesth Analg. 1997;85:248–51.
11. Dowd NP, Karski JM, Cheng DC, Carroll JA, Lin Y, James RL, et al. Pharmacokinetics of tranexamic acid during cardiopulmonary bypass. Anesthesiology. 2002;97:390–9.
12. Mangano DT, Tudor IC, Dietzel C, Multicenter Study of Perioperative Ischemia Research G, Ischemia Research, Education F. The risk associated with aprotinin in cardiac surgery. N Engl J Med. 2006;354:353–65.
13. Mangano DT, Miao Y, Vuylsteke A, Tudor IC, Juneja R, Filipescu D, et al. Mortality associated with aprotinin during 5 years following coronary artery bypass graft surgery. JAMA. 2007;297:471–9.
14. Aoki N, Sakata Y, Ichinose A. Fibrin-associated plasminogen activation in alpha 2-plasmin inhibitor deficiency. Blood. 1983;62:1118–22.
15. Ichinose A, Aoki N. The initiation of fibrinolysis in alpha 2-plasmin inhibitor deficient plasma. Role of fibrin. Thromb Res. 1986;41:847–54.
16. Booth NA, Simpson AJ, Croll A, Bennett B, MacGregor IR. Plasminogen activator inhibitor (PAI-1) in plasma and platelets. Br J Haematol. 1988;70:327–33.
17. Robbie LA, Booth NA, Croll AM, Bennett B. The roles of alpha 2-antiplasmin and plasminogen activator inhibitor 1 (PAI-1) in the inhibition of clot lysis. Thromb Haemost. 1993;70:301–6.
18. Sakata Y, Aoki N. Cross-linking of alpha 2-plasmin inhibitor to fibrin by fibrin-stabilizing factor. J Clin Invest. 1980;65:290–7.
19. Aoki N, Saito H, Kamiya T, Koie K, Sakata Y, Kobakura M. Congenital deficiency of alpha 2-plasmin inhibitor associated with severe hemorrhagic tendency. J Clin Invest. 1979;63:877–84.
20. Bajzar L, Morser J, Nesheim M. TAFI, or plasma procarboxypeptidase B, couples the coagulation and fibrinolytic cascades through the thrombin–thrombomodulin complex. J Biol Chem. 1996;271:16603–8.
21. Felez J, Chanquia CJ, Fabregas P, Plow EF, Miles LA. Competition between plasminogen and tissue plasminogen activator for cellular binding sites. Blood. 1993;82:2433–41.
22. Schneider M, Boffa M, Stewart R, Rahman M, Koschinsky M, Nesheim M. Two naturally occurring variants of TAFI (Thr-325 and Ile-325) differ substantially with respect to thermal stability and antifibrinolytic activity of the enzyme. J Biol Chem. 2002;277:1021–30.
23. Sakharov DV, Nagelkerke JF, Rijken DC. Rearrangements of the fibrin network and spatial distribution of fibrinolytic components during plasma clot lysis. J Biol Chem. 1996; 271:2133–8.
24. Levin EG, Marzec U, Anderson J, Harker LA. Thrombin stimulates tissue plasminogen activator release from cultured human endothelial cells. J Clin Invest. 1984;74:1988–95.
25. Chandler WL, Levy WC, Veith RC, Stratton JR. A kinetic model of the circulatory regulation of tissue plasminogen activator during exercise, epinephrine infusion, and endurance training. Blood. 1993;81:3293–302.
26. Wall U, Jern S, Tengborn L, Jern C. Evidence of a local mechanism for desmopressin-induced tissue-type plasminogen activator release in human forearm. Blood. 1998;91:529–37.
27. Emeis JJ. Regulation of the acute release of tissue-type plasminogen activator from the endothelium by coagulation activation products. Ann NY Acad Sci. 1992;667:249–58.
28. Tanaka K, Morimoto T, Yada I, Kusagawa M, Deguchi K. Physiologic role of enhanced fibrinolytic activity during cardiopulmonary bypass in open heart surgery. ASAIO Trans. 1987;33:505–9.
29. Journois D, Mauriat P, Pouard P, Marchot P, Amiral J, Safran D. Assessment of coagulation factor activation during cardiopulmonary bypass with a new monoclonal antibody. J Cardiothorac Vasc Anesth. 1994;8:157–61.
30. Kang H-M, Kalnoski MH, Frederick M, Chandler WL. The kinetics of plasmin inhibition by aprotinin in vivo. Thromb Res. 2005;115:327–40.
31. Harker LA, Malpass TW, Branson HE, Hessel EA 2nd, Slichter SJ. Mechanism of abnormal bleeding in patients undergoing cardiopulmonary bypass: acquired transient platelet dysfunction associated with selective alpha-granule release. Blood. 1980;56:824–34.
32. Weinstein M, Ware J, Troll J, Salzman E. Changes in von Willebrand factor during cardiac surgery: effect of desmopressin acetate. Blood. 1988;71:1648–55.
33. Rondaij MG, Bierings R, Kragt A, van Mourik JA, Voorberg J. Dynamics and plasticity of Weibel–Palade bodies in endothelial cells. Arterioscler Thromb Vasc Biol. 2006;26:1002–7.
34. Tracy RP, Rubin DZ, Mann KG, Bovill EG, Rand M, Geffken D, et al. Thrombolytic therapy and proteolysis of factor V. J Am Coll Cardiol. 1997;30:716–24.
35. Nogami K, Shima M, Matsumoto T, Nishiya K, Tanaka I, Yoshioka A. Mechanisms of plasmin-catalyzed inactivation of factor VIII: a crucial role for proteolytic cleavage at Arg336 responsible for plasmin-catalyzed factor VIII inactivation. J Biol Chem. 2007;282:5287–95.

36. Cramer EM, Lu H, Caen JP, Soria C, Berndt MC, Tenza D. Differential redistribution of platelet glycoproteins Ib and IIb-IIIa after plasmin stimulation. Blood. 1991;77:694–9.
37. Quinton TM, Kim S, Derian CK, Jin J, Kunapuli SP. Plasmin-mediated activation of platelets occurs by cleavage of protease-activated receptor 4. J Biol Chem. 2004;279:18434–9.
38. Bidstrup BP, Royston D, Sapsford RN, Taylor KM. Reduction in blood loss and blood use after cardiopulmonary bypass with high dose aprotinin (Trasylol). J Thorac Cardiovasc Surg. 1989;97:364–72.
39. Horrow JC, Hlavacek J, Strong MD, Collier W, Brodsky I, Goldman SM, et al. Prophylactic tranexamic acid decreases bleeding after cardiac operations. J Thorac Cardiovasc Surg. 1990;99:70–4.
40. Kuitunen A, Hiippala S, Vahtera E, Rasi V, Salmenpera M. The effects of aprotinin and tranexamic acid on thrombin generation and fibrinolytic response after cardiac surgery. Acta Anaesthesiol Scand. 2005;49:1272–9.
41. Levi M, Cromheecke ME, de Jonge E, Prins MH, de Mol BJ, Briet E, et al. Pharmacological strategies to decrease excessive blood loss in cardiac surgery: a meta-analysis of clinically relevant endpoints. Lancet. 1999;354:1940–7.
42. Sedrakyan A, Treasure T, Elefteriades JA. Effect of aprotinin on clinical outcomes in coronary artery bypass graft surgery: a systematic review and meta-analysis of randomized clinical trials. J Thorac Cardiovasc Surg. 2004;128:442–8.
43. van der Linden J, Lindvall G, Sartipy U. Aprotinin decreases postoperative bleeding and number of transfusions in patients on clopidogrel undergoing coronary artery bypass graft surgery: a double-blind, placebo-controlled, randomized clinical trial. Circulation. 2005;112:I276–80.
44. Despotis GJ, Joist JH, Hogue CW, Alsoufiev A, Joiner-Maier D, Santoro SA, et al. More effective suppression of hemostatic system activation in patients undergoing cardiac surgery by heparin dosing based on heparin blood concentrations rather than ACT. Thromb Haemost. 1996;76:902–8.
45. Levy JH, Despotis GJ, Szlam F, Olson P, Meeker D, Weisinger A. Recombinant human transgenic antithrombin in cardiac surgery: a dose-finding study. Anesthesiology. 2002;96:1095–102.
46. Langdown J, Johnson DJD, Baglin TP, Huntington JA. Allosteric activation of antithrombin critically depends upon hinge region extension. J Biol Chem. 2004;279:47288–97.
47. Dietrich W, Spannagl M, Schramm W, Vogt W, Barankay A, Richter JA. The influence of preoperative anticoagulation on heparin response during cardiopulmonary bypass. J Thorac Cardiovasc Surg. 1991;102:505–14.
48. Staples MH, Dunton RF, Karlson KJ, Leonardi HK, Berger RL. Heparin resistance after preoperative heparin therapy or intra-aortic balloon pumping. Ann Thorac Surg. 1994;57:1211–6.
49. Warkentin TE. Heparin-induced thrombocytopenia: IgG-mediated platelet activation, platelet microparticle generation, and altered procoagulant/anticoagulant balance in the pathogenesis of thrombosis and venous limb gangrene complicating heparin-induced thrombocytopenia. Transfus Med Rev. 1996;10:249–58.
50. Korte W, Gabi K, Rohner M, Gahler A, Szadkowski C, Schnider TW, et al. Preoperative fibrin monomer measurement allows risk stratification for high intraoperative blood loss in elective surgery. Thromb Haemost. 2005;94:211–5.
51. Suzuki S, Matsuo T, Kobayashi H, Matsuo M, Shimamo C, Koide M, et al. Antithrombotic treatment (argatroban vs. heparin) in coronary angioplasty in angina pectoris: effects on inflammatory, hemostatic, and endothelium-derived parameters. Thromb Res. 2000;98:269–79.
52. Minnema MC, ten Cate H, van Beek EJ, van den Ende A, Hack CE, Brandjes DP. Effects of heparin therapy on fibrinolysis in patients with pulmonary embolism. Thromb Haemost. 1997;77:1164–7.
53. Nieuwenhuizen W, Bos R. Soluble fibrin and degradation products of fibrinogen (FgDP), fibrin (FbDP; D-dimer) and total of FgDP and FbDP (TDP). In: Jespersen J, Bertina RM, Haverkate F, editors. Laboratory techniques in thrombosis—a manual. 2/e ed. Dordrecht: Kluwer Academic Publishers; 2000. p. 275–84.
54. Ferrucci L, Corsi A, Lauretani F, Bandinelli S, Bartali B, Taub DD, et al. The origins of age-related proinflammatory state. Blood. 2005;105:2294–9.
55. Royston D. Intraoperative coronary thrombosis: can aprotinin be incriminated? J Cardiothorac Vasc Anesth. 1994;8:137–41.
56. Alderman EL, Levy JH, Rich JB, Nili M, Vidne B, Schaff H, et al. Analyses of coronary graft patency after aprotinin use: results from the International Multicenter Aprotinin Graft Patency Experience (IMAGE) trial. J Thorac Cardiovasc Surg. 1998;116:716–30.
57. Mangano DT, Multicenter Study of Perioperative Ischemia Research G. Aspirin and mortality from coronary bypass surgery. N Engl J Med. 2002;347:1309–17.
58. Aronson S, Fontes ML, Miao Y, Mangano DT, Investigators of the Multicenter Study of Perioperative Ischemia Research G, Ischemia Research, Education F. Risk index for perioperative renal dysfunction/failure: critical dependence on pulse pressure hypertension. Circulation. 2007;115:733–42.
59. Yamamoto K, Loskutoff DJ. Fibrin deposition in tissues from endotoxin-treated mice correlates with decreases in the expression of urokinase-type but not tissue-type plasminogen activator. J Clin Invest. 1996;97:2440–51.
60. Rosenberg RD. Vascular-bed-specific hemostasis and hypercoagulable states: clinical utility of activation peptide assays in predicting thrombotic events in different clinical populations. Thromb Haemost. 2001;86:41–50.
61. Carmeliet P, Schoonjans L, Kieckens L, Ream B, Degen J, Bronson R, et al. Physiological consequences of loss of plasminogen activator gene function in mice. Nature. 1994;368:419–24.
62. Krishnamurti C, Bolan C, Colleton CA, Reilly TM, Alving BM. Role of plasminogen activator inhibitor-1 in promoting fibrin deposition in rabbits infused with ancrod or thrombin. Blood. 1993;82:3631–6.
63. Lane DA, Philippou H, Huntington JA. Directing thrombin. Blood. 2005;106:2605–12.
64. Muller-Berghaus G, Roka L, Lasch HG. Induction of glomerular microclot formation by fibrin monomer infusion. Thromb Diath Haemorrh. 1973;29:375–83.
65. Aird WC. Phenotypic heterogeneity of the endothelium: I. Structure, function, and mechanisms. Circ Res. 2007;100:158–73.
66. Aird WC. Phenotypic heterogeneity of the endothelium: II. Representative vascular beds. Circ Res. 2007;100:174–90.
67. Giles AR, Nesheim ME, Herring SW, Hoogendoorn H, Stump DC, Heldebrant CM. The fibrinolytic potential of the normal primate following the generation of thrombin in vivo. Thromb Haemost. 1990;63:476–81.
68. Taylor FB Jr, Hoogendoorn H, Chang AC, Peer G, Nesheim ME, Catlett R, et al. Anticoagulant and fibrinolytic activities are promoted, not retarded, in vivo after thrombin generation in the presence of a monoclonal antibody that inhibits activation of protein C. Blood. 1992;79:1720–8.
69. Hanson SR, Griffin JH, Harker LA, Kelly AB, Esmon CT, Gruber A. Antithrombotic effects of thrombin-induced activation of endogenous protein C in primates. J Clin Invest. 1993;92:2003–12.
70. Esmon CT. The protein C pathway. Chest. 2003;124:26S–32S.

71. Sakata Y, Curriden S, Lawrence D, Griffin JH, Loskutoff DJ. Activated protein C stimulates the fibrinolytic activity of cultured endothelial cells and decreases antiactivator activity. Proc Natl Acad Sci USA. 1985;82:1121–5.
72. Nicolaes GAF, Dahlback B. Factor V and thrombotic disease: description of a Janus-faced protein. Arterioscler Thromb Vasc Biol. 2002;22:530–8.
73. Sniecinski R, Szlam F, Chen EP, Bader SO, Levy JH, Tanaka KA. Antithrombin deficiency increases thrombin activity after prolonged cardiopulmonary bypass. Anesth Analg. 2008;106:713–8.
74. Jordan RE, Nelson RM, Kilpatrick J, Newgren JO, Esmon PC, Fournel MA. Inactivation of human antithrombin by neutrophil elastase. Kinetics of the heparin-dependent reaction. J Biol Chem. 1989;264:10493–500.
75. Drake TA, Ruf W, Morrissey JH, Edgington TS. Functional tissue factor is entirely cell surface expressed on lipopolysaccharide-stimulated human blood monocytes and a constitutively tissue factor-producing neoplastic cell line. J Cell Biol. 1989;109:389–95.
76. Moore KL, Esmon CT, Esmon NL. Tumor necrosis factor leads to the internalization and degradation of thrombomodulin from the surface of bovine aortic endothelial cells in culture. Blood. 1989;73:159–65.
77. Gando S, Kameue T, Nanzaki S, Nakanishi Y. Cytokines, soluble thrombomodulin and disseminated intravascular coagulation in patients with systemic inflammatory response syndrome. Thromb Res. 1995;80:519–26.
78. Tanaka KA, Sniecinski R. Systemic thromboses after cardiopulmonary bypass: is it thrombin or antithrombin? Anesthesiology. 2006;105:428.
79. Brummel KE, Paradis SG, Butenas S, Mann KG. Thrombin functions during tissue factor-induced blood coagulation. Blood. 2002;100:148–52.
80. Kuharsky AL, Fogelson AL. Surface-mediated control of blood coagulation: the role of binding site densities and platelet deposition. Biophys J. 2001;80:1050–74.
81. Shore-Lesserson L, Reich DL. A case of severe diffuse venous thromboembolism associated with aprotinin and hypothermic circulatory arrest in a cardiac surgical patient with factor V Leiden. Anesthesiology. 2006;105:219–21.
82. Lichtman AD, Carullo V, Minhaj M, Karkouti K. Case 6-2007: massive intraoperative thrombosis and death after recombinant activated factor VII administration. J Cardiothorac Vasc Anesth. 2007;21:897–902.
83. Dusel CH, Grundmann C, Eich S, Seitz R, Konig H. Identification of prothrombin as a major thrombogenic agent in prothrombin complex concentrates. Blood Coagul Fibrinolysis. 2004;15:405–11.
84. Szlam F, Taketomi T, Sheppard CA, Kempton CL, Levy JH, Tanaka KA. Antithrombin affects hemostatic response to recombinant activated factor VII in factor VIII deficient plasma. Anesth Analg. 2008;106:719–24.
85. Sniecinski RM, Chen EP, Tanaka KA. Reduced levels of fibrin (antithrombin I) and antithrombin III underlie coagulopathy following complex cardiac surgery. Blood Coagul Fibrinolysis. 2008;19:178–9.
86. Levy JH, Adkinson NF Jr. Anaphylaxis during cardiac surgery: implications for clinicians. Anesth Analg. 2008;106:392–403.
87. Ratnoff OD. Epsilon aminocaproic acid—a dangerous weapon. N Engl J Med. 1969;280:1124–5.
88. Charytan C, Purtilo D. Glomerular capillary thrombosis and acute renal failure after epsilon-amino caproic acid therapy. N Engl J Med. 1969;280:1102–4.
89. Garcha PS, Mohan CVR, Sharma RM. Death after an inadvertent intrathecal injection of tranexamic acid. Anesth Analg. 2007;104:241–2.
90. Martin K, Wiesner G, Breuer T, Lange R, Tassani P. The risks of aprotinin and tranexamic acid in cardiac surgery: a one-year follow-up of 1188 consecutive patients. Anesth Analg. 2008;107:1783–90.
91. Furtmuller R, Schlag MG, Berger M, Hopf R, Huck S, Sieghart W, et al. Tranexamic acid, a widely used antifibrinolytic agent, causes convulsions by a gamma-aminobutyric acid(A) receptor antagonistic effect. J Pharmacol Exp Ther. 2002;301:168–73.
92. Levi M, Hovingh K, Cannegieter SC, Vermeulen M, Buller HR, Rosendaal FR. Bleeding in patients receiving vitamin K antagonists who would have been excluded from trials on which the indication for anticoagulation was based. Blood. 2008;111:4471–6.
93. Casati V, Gerli C, Franco A, Della Valle P, Benussi S, Alfieri O, et al. Activation of coagulation and fibrinolysis during coronary surgery: on-pump versus off-pump techniques. Anesthesiology. 2001;95:1103–9.
94. Bolliger D, Szlam F, Molinaro RJ, Rahe-Meyer N, Levy JH, Tanaka KA. Finding the optimal concentration range for fibrinogen replacement after severe haemodilution: an in vitro model. Br J Anaesth. 2009;102:793–9.
95. Ganter MT, Hofer CK. Coagulation monitoring: current techniques and clinical use of viscoelastic point-of-care coagulation devices. Anesth Analg. 2008;106:1366–75.
96. Tanaka KA, Key NS, Levy JH. Blood coagulation: hemostasis and thrombin regulation. Anesth Analg. 2009;108:1433–46.
97. Sakharov DV, Rijken DC. Superficial accumulation of plasminogen during plasma clot lysis. Circulation. 1995;92:1883–90.
98. Blome M, Isgro F, Kiessling AH, Skuras J, Haubelt H, Hellstern P, et al. Relationship between factor XIII activity, fibrinogen, haemostasis screening tests and postoperative bleeding in cardiopulmonary bypass surgery. Thromb Haemost. 2005;93:1101–7.
99. Carroll RC, Chavez JJ, Snider CC, Meyer DS, Muenchen RA. Correlation of perioperative platelet function and coagulation tests with bleeding after cardiopulmonary bypass surgery. J Lab Clin Med. 2006;147:197–204.
100. Pelletier JPR, Transue S, Snyder EL. Pathogen inactivation techniques. Baillieres Best Pract Res Clin Haematol. 2006; 19:205–42.
101. Levy JH, Tanaka KA, Dietrich W. Perioperative hemostatic management of patients treated with vitamin K antagonists. Anesthesiology. 2008;109:905–17.
102. Chowdhury P, Saayman AG, Paulus U, Findlay GP, Collins PW. Efficacy of standard dose and 30 ml/kg fresh frozen plasma in correcting laboratory parameters of haemostasis in critically ill patients. Br J Haematol. 2004;125:69–73.
103. Triulzi DJ. Transfusion-related acute lung injury: current concepts for the clinician. Anesth Analg. 2009;108:770–6.
104. Diprose P, Herbertson MJ, O'Shaughnessy D, Gill RS. Activated recombinant factor VII after cardiopulmonary bypass reduces allogeneic transfusion in complex non-coronary cardiac surgery: randomized double-blind placebo-controlled pilot study. Br J Anaesth. 2005;95:596–602.
105. Romagnoli S, Bevilacqua S, Gelsomino S, Pradella S, Ghilli L, Rostagno C, et al. Small-dose recombinant activated factor VII (NovoSeven) in cardiac surgery. Anesth Analg. 2006;102:1320–6.
106. O'Connell KA, Wood JJ, Wise RP, Lozier JN, Miles Braun M. Thromboembolic adverse events after use of recombinant human coagulation factor VIIa. JAMA. 2006;295:293–8.
107. Dietrich W, Nicklisch S, Koster A, Spannagl M, Giersiefen H, van de Locht A. CU-2010-a novel small molecule protease inhibitor with antifibrinolytic and anticoagulant properties. Anesthesiology. 2009;110:123–30.