CARDIOPULMONARY SUPPORT AND PHYSIOLOGY

SELECTIVE ANTICOAGULATION WITH ACTIVE SITE-BLOCKED FACTOR IXA SUGGESTS SEPARATE ROLES FOR INTRINSIC AND EXTRINSIC COAGULATION PATHWAYS IN CARDIOPULMONARY BYPASS

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Background: Multiple stimuli converge in cardiopulmonary bypass to create a tremendous prothrombotic stimulus. The ideal anticoagulant for cardiopulmonary bypass should selectively target only the intravascular stimuli, thereby eliminating pathologic clotting in the bypass circuit while preserving hemostasis in the thoracic cavity. We propose the inhibition of factor IX as such a targeted anticoagulant strategy. **Methods:** We prepared an inhibitor of activated factor IX and applied it to a primate model of cardiopulmonary bypass to confirm the anticoagulant efficacy of activated factor IX in this setting and to assess more subtle markers of thrombin generation, macrophage procoagulant activity, and cellular tissue factor expression. Seven baboons that received activated factor IX (460 µg/kg) and 7 that received heparin (300 IU/kg) and protamine underwent cardiopulmonary bypass for 90 minutes and were followed after the operation for 3 hours. **Results:** Analysis of plasma factor IX activity demonstrated adequate inhibition (<20%) of factor IX throughout cardiopulmonary bypass. Activated factor IX-treated baboons demonstrated similar circuit patency to heparin-treated baboons but had significantly diminished intraoperative blood loss. Preservation of extravascular hemostasis was further demonstrated in activated factor IX-treated animals by (1) significantly increased levels of thrombin-antithrombin III complex and prothrombin activation peptide (F₁₊₂) without intravascular thrombosis, (2) significantly greater macrophage procoagulant activity in pericardial-derived monocytes, and (3) immunohistochemical evidence of tissue factor expression in pericardial mesothelial cells and macrophages. **Conclusions:** Anticoagulation with activated factor IX allows for intravascular anticoagulation with maintenance of extravascular hemostasis. These findings suggest activated factor IX as an agent that not only exemplifies a targeted approach to selective anticoagulation in cardiac surgery but also further characterizes the procoagulant milieu during cardiopulmonary bypass. (J Thorac Cardiovasc Surg 1998;116:860-9)

Although the mechanism by which coagulation is activated in the setting of cardiopulmonary bypass (CPB) remains to be elucidated fully, multiple factors

are thought to converge that comprise a tremendous, complex prothrombotic stimulus.¹⁻⁴ The contributions of 3 separate procoagulant stimuli have been most

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We prepared a novel inhibitor of activated factor IX (IXai) and have used this agent as an anticoagulant in a canine model of CPB¹⁸ in which we demonstrated the feasibility of this anticoagulant strategy. In the current study, we have extended this evaluation to a primate model to confirm the anticoagulant efficacy of IXai with assessment of bypass circuit patency and surgical hemostasis and to assess more subtle markers of thrombin generation, macrophage procoagulant activity, and cellular TF expression. These findings suggest IXai as a novel agent that not only exemplifies a targeted approach to selective anticoagulation in cardiac surgery but also further characterizes the procoagulant milieu during CPB.

IXai

The diagram illustrates the blood clotting cascade, showing the convergence of the Intrinsic and Extrinsic systems into a common pathway.

Intrinsic System: IX $\rightarrow$ IXa

Extrinsic System: VIIa / TF

Common Pathway:

- IXa and VIIa / TF converge to activate X (labeled *low TF* and *high TF*).
- X $\rightarrow$ Xa
- Xa, along with Va, converts prothrombin to thrombin.
- Thrombin converts Fibrinogen to Fibrin, which forms the Clot.

The diagram also shows the activation of VIIIa by IXa and the activation of X by VIIIa and Xa.

Primate model

All animals received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the

National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication 85-23, revised 1985). This study was approved by the Columbia University Institutional Animal Review Committee and was conducted according to policies set forth by Columbia University.

Efficacy of anticoagulation

Analysis of blood samples. Routine hematologic analysis was performed before the initiation of CPB, every 30 minutes during CPB, and after the operation to determine levels of hemoglobin and hematocrit (analyzed on an ISTAT arterial blood analyzer [I-STAT Corporation, Princeton, NJ]), prothrombin time, activated partial thromboplastin time (measured on the ST4 Bio clot detection system with reagents provided by the manufacturer [Diagnostica Stago, Asnieres, France]), and activated clotting time (measured on a Hemochron model 801 ACT machine [International Technidyne Corp, Edison, NJ] with Flip-Top Celite ACT tubes [Celite Corporation, Quincy, Mass]).

Assessment of IXai antithrombotic effect. The antithrombotic activity of factor IXai in plasma was assessed by the testing of peripheral plasma with an assay of factor IX activity as determined on the ST4 Bio clot detection system (Diagnostica Stago) with factor IX-deficient plasma, a partial thromboplastin time reagent, and 0.025 mmol/L calcium chloride (Diagnostica Stago) in the manner described by the manufacturer. A standard curve for factor IX activity was established with factor control plasma (Diagnostica Stago). Data are expressed both as an absolute value in seconds and as a percent of normal factor IX activity.

Assessment of fibrin deposition in the CPB tubing and filters. At the end of CPB, the tubing and filters were removed and subjected to qualitative analysis by scanning electron microscopy as previously described.²³

Assessment of extrinsic hemostasis. To establish the effects of IXai on bleeding tendency outside the operative field, a standardized incisional model in the abdomen was used, as described previously.²⁴ A uniform 1-cm deep, 5-cm long abdominal wall incision was made before the operation, during CPB, and 3 hours after the operation. A preweighed gauze pad was inserted for 5 minutes. At the end of that time, the gauze was removed and weighed to quantitate the blood loss.

Thrombin generation

Assessment of peripheral markers of thrombin generation. Blood was obtained from the peripheral arterial catheter at baseline, after infusion of IXai, and at designated times on CPB (0.5 hour, 1 hour, and 1.5 hours). Blood was also obtained from the pericardial cavity, drawn from the CPB circuit after infusion of IXai and at the times mentioned earlier during CPB. Blood was drawn into sodium citrate (3.8%; 9:1 vol/vol), and platelet-poor plasma was separated and assayed by enzyme-linked immunosorbent assay (ELISA) for thrombin-antithrombin III complex (TAT) and prothrombin activation peptide (F₁₊₂) as instructed by the manufacturer (Behring Diagnostics, Inc, Westwood, Mass). The limits of detection in these assays were as follows: TAT, 2 µg/L; F₁₊₂, 0.04 nmol/L.

Macrophage procoagulant activity

Isolation of mononuclear cells and analysis of procoagulant activity. Immediately before the animals were killed, blood (~18 mL) was retrieved from the arterial catheter, bypass circuit, and pericardial cavity from 6 animals (3 IXai, 3 heparin). In addition, adherent mononuclear cells were harvested from the arterial oxygenator when the surface was washed in a solution containing EDTA (0.01 mol/L), NaCl (0.150 mol/L), and HEPES (0.01 mol/L). Cells were then concentrated by centrifugation (900g for 20 minutes); the resulting pellet was resuspended in Hanks' balanced salt solution (Gibco, Grand Island, NY) containing HEPES (0.025 mol/L; pH 7.4). In all cases, samples containing mononuclear cells were then layered onto a histopaque gradient (1077; Sigma Chemical Co, St Louis, Mo) for isolation of mononuclear cell fraction as previously described.²⁵ The resultant mononuclear cells (2.5×10^7 cells/assay) were placed into 24-well tissue culture plates (Corning, Inc, Corning, NY) and incubated alone or in the presence of lipopolysaccharide (10 µg/mL) from *Escherichia coli* serotype 026:B6 (Sigma Chemical Co) in Hanks' buffered salt solution containing human plasma (20%) for 2 hours at 37°C. Analysis of TF activity was performed as previously described.^{8,26,27} In brief, procoagulant activity was performed with a 1-step recalcification time procedure. Mononuclear cells were lysed by the addition of octyl-β-glucopyranoside (15 mmol/L; Calbiochem) in Hanks' balanced salt solution containing HEPES (0.025 mol/L). The resulting suspension was incubated with pooled normal plasma at 37°C for 3 minutes, after which time CaCl₂ (0.025 mol/L) was added; the clotting time was determined by visual detection of clot formation. Each sample was run in triplicate, and serial dilutions of purified human TF (American Diagnostica, Greenwich, Conn) were used to generate a standard curve.

Cellular TF expression

Immunohistochemistry. Pericardial tissue was harvested at initiation of CPB and after 90 minutes and placed in formalin (10%). Paraffin sections (5-µm thick) were prepared and stained either with anti-TF immunoglobulin G (IgG) (1:100 dilution; American Diagnostica) or nonimmune IgG (mouse) according to the manufacturer's instructions.

Statistical analysis. Data are expressed as the mean ± the SE in the text and as mean ± SD in the tables and figures, as indicated. Serial data from each group were evaluated by the repeated measures analysis of variance and then by paired *t* test.

Results

Efficacy of anticoagulation

Analysis of blood samples. The use of IXai as an anticoagulant in CPB did not adversely affect routinely measured hematologic values. Compared with heparin, no untoward effects were observed at 90 minutes of CPB on either white blood cell count (H, $4.7 \pm 1.1 \times 10^3$, versus IXai, $5.2 \pm 0.9 \times 10^3$; *P* = .3), hematocrit values (H, $17.2\% \pm 2.1\%$, versus IXai, $18.9\% \pm 2.4\%$;

Table I. Analysis of coagulation variables and factor IX activity in baboons undergoing CPB

	ACT (sec)	PT (sec)	PTT (sec)	Factor IX activity	
				Seconds	Percent
Heparin					
Baseline	136 ± 17	15.6 ± 0.3	36.7 ± 2.3	31 ± 1.1	>100
CPB	570 ± 87	17 ± 0.7	>180	>180	<6
90 min CPB	780 ± 220	16.9 ± 0.6	>180	>180	<6
60 min after CPB	150 ± 18	15 ± 0.8	54 ± 3.2	56 ± 2.1	56
120 min after CPB	143 ± 17	15.6 ± 0.7	52 ± 4.6	50 ± 1.3	76
180 min after CPB	135 ± 22	16 ± 0.8	46 ± 5.2	48 ± 2.4	96
IXai					
Baseline	117 ± 7	15 ± 0.6	38 ± 1.9	31 ± 2.3	>100
CPB	154 ± 17	16 ± 0.6	67 ± 2.3	69 ± 2.1	32
90 min CPB	165 ± 23	16.5 ± 0.4	74 ± 3.4	76 ± 2.1	19
60 min after CPB	143 ± 35	17 ± 0.6	56 ± 2.3	57 ± 3.2	66
120 min after CPB	134 ± 22	16.6 ± 0.7	54 ± 4.1	55 ± 3.1	73
180 min after CPB	120 ± 18	15.8 ± 0.7	49 ± 5.2	52 ± 3.1	86

P < .05, comparing heparin to IXai for CPB and 90-minute values: ACT, Activated clotting time; PT, prothrombin time; PTT, (PT), partial thromboplastin time.

Table II. Thoracic cavity blood loss in baboons undergoing CPB

	Total blood loss (mL)	Incisional wound model (g)		
		Baseline	CPB	After CPB
Heparin	1250 ± 115	0.9 ± 0.2	5.9 ± 0.7	1.4 ± 0.4
IXai	480 ± 30*	0.8 ± 0.4	1.1 ± 0.5*	0.9 ± 0.3

**P* < .05 by ANOVA, comparing IXai versus heparin.

P = .4), or platelet counts (H, $1.5 \pm 0.2 \times 10^9/L$, versus IXai, $1.9 \pm 0.6 \times 10^9/L$; *P* = .3); both groups demonstrated similar decreases in these values consistent with hemodilution. Standard parameters of coagulation, used to assess and quantitate antithrombotic effect in the peripheral plasma, showed that, compared with heparin-treated baboons, baboons treated with IXai demonstrated significantly decreased rises in both prothrombin time and activated partial thromboplastin time. Similarly, compared with heparin-treated baboons in which activated clotting time (ACT) was maintained for more than 480 seconds throughout CPB, doses of IXai that effectively prevented thrombosis within the extracorporeal circulation were associated with ACTs that did not differ from baseline (Table I).

Assessment of IXai antithrombotic effect. Using an assay assessing in vivo factor IX activity, we determined that normal factor IX activity in baboons is approximately 31 seconds (>100% factor IX activity). At effective antithrombotic doses of IXai, the factor IX activity (69 ± 2.1 seconds) represented less than 35% factor IX activity and was maintained at that level

Table III. Analysis of macrophage procoagulant activity (PCA) in baboons undergoing CPB with IXai

	Activity (%)	
	(-)LPS	(+)LPS
Intravascular space		
Peripheral blood	0	21 ± 3
Arterial filter	0	20 ± 2
CPB circuit	0	22 ± 3
Extravascular space		
Pericardial blood	56 ± 4*	99 ± 1†

LPS, Lipopolysaccharide.

**P* < .05.

†*P* < .001 by ANOVA, comparing extravascular versus intravascular values.

through 90 minutes of CPB (Table I). Assessment of factor IX activity beyond CPB revealed that levels slowly rose by 1 hour after CPB (57 ± 3.2 seconds; 66% activity), and further increased by 3 hours after initiation of CPB (52 ± 3.1 seconds; 86% activity). In primate-clearance studies, IXai demonstrates an in vivo half life of approximately 90 minutes with factor IX activity levels returning to more than 100% by 4 hours after administration. Factor IX activity in heparin-treated animals was less than 6% throughout CPB and returned to more than 100% activity after heparin reversal with protamine.

Assessment of fibrin deposition in the CPB tubing and filters. Compared with baboons receiving heparin/protamine, baboons treated with IXai demonstrated no evidence of increased pressures within the CPB circuitry. Consistent with these findings, scanning electron microscopy of the arterial filters retrieved at

Fig 2. Scanning electron microscopy of the CPB filters. CPB performed in animals treated with factor IXai was associated with comparable clinically inapparent fibrin deposition on arterial filters (*left panel*) when compared with animals treated with heparin (*right panel*). Original magnification is indicated.

the end of CPB revealed no evidence of increased clot formation in baboons treated with effective antithrombotic doses of IXai (460 $\mu\text{g/kg}$) compared with baboons treated with heparin (Fig 2).

Assessment of extrinsic hemostasis. Baboons undergoing CPB with IXai demonstrated significantly diminished blood loss at the end of CPB and during the postoperative period, when compared with baboons receiving heparin/protamine (480 ± 30 mL versus 1250 ± 115 mL; $P < .05$, respectively; Table II). Importantly, at effective antithrombotic doses of IXai, clot formation was noted along the cut surface of the sternum and surgical tissue planes throughout the thoracic cavity. Furthermore, evaluation of the effects of IXai on bleeding tendency outside the operative field with a standardized incisional abdominal wound model demonstrated that, at effective antithrombotic doses of IXai,

there was no evidence of an enhanced bleeding tendency either during CPB or after the operation compared with baseline (Table II). In contrast, significantly increased bleeding was observed after infusion of heparin during CPB, which returned to baseline after heparin reversal with protamine.

Thrombin generation

Assessment of peripheral markers of thrombin generation. Evaluation of thrombin generation in peripheral plasma showed that, compared with heparin-treated animals, animals treated with IXai demonstrated significantly increased levels of TAT and prothrombin activation peptide (F_{1+2}), with peak levels occurring on CPB, although levels of both markers rose progressively in the postoperative period (Fig 3). (Both TAT and F_{1+2} are sensitive/specific markers of the effect of factor Xa on prothrombin.) Evaluation of TAT and F_{1+2} in peri-

cardial blood after 90 minutes of CPB demonstrated an almost 4-fold increased rise when compared with concurrently obtained peripheral blood samples in IXai-treated animals (TAT, 423 ± 47 $\mu\text{g/L}$ versus 157 ± 78 $\mu\text{g/L}$; $P < .002$; F_{1+2} , 26 ± 8 nmol/L versus 6 ± 2 nmol/L; $P < .001$).

Macrophage procoagulant activity

Isolation of mononuclear cells and analysis of procoagulant activity. Consistent with the hypothesis that activation of the extrinsic pathway largely occurs in the surgical wound, retrieval of monocytes from intravascular space (peripheral blood, arterial filter, and CPB pump) during the procedure revealed no demonstrable TF activity (Table III). In contrast, monocytes isolated from extravascular space (pericardial blood) manifested significantly greater procoagulant activity compared with those in the circuit/periphery ($P < .05$). Lipopolysaccharide, a known inducer of monocyte procoagulant activity, was used as a positive control in these assays.

Cellular TF expression

Immunohistochemistry. Further evidence for enhanced TF activity in the surgical wound was demonstrated by immunohistochemical examination of the pericardium during CPB (approximately 1 hour after sternotomy and pericardial dissection). Striking TF expression in the pericardial mesothelial cells and in the vasculature and macrophages that pervade pericardial tissue (Fig 4, A through D) was demonstrated with anti-TF IgG. In contrast, staining of an adjacent section with nonimmune IgG was negative (Fig 4, D).

Discussion

The relative contributions of the different coagulation pathways in the potent, prothrombotic environment of CPB have not yet been defined.¹⁻⁴ The intrinsic pathway has been implicated most often as the result of contact of blood with the extensive foreign surface of the extracorporeal circuit. In support of this, reduced levels of factor XII, and increased levels of its activated form (XIIa), have been demonstrated during CPB.^{27,28} Furthermore, peripheral blood mononuclear cells have been shown to be adsorbed on the CPB circuit where they become activated to express low levels of TF, thereby contributing to the overall procoagulant response.⁶ A role for the extrinsic pathway in CPB has also been suggested. In a study by Burman and colleagues,⁵ a sharp rise in markers of thrombin generation was noted in a patient with factor XII deficiency who was undergoing CPB, suggesting coagulation activation apart from the intrinsic system. Additionally, Chung and colleagues⁸ have reported enhanced expression of TF in monocytes isolated from pericardial blood

Fig 3. Analysis of thrombin generation in baboons undergoing CPB. Baboons underwent CPB with either IXai (solid line) or heparin/protamine (dashed line). **A**, At the indicated times, blood was withdrawn from a peripheral arterial line for analysis by ELISA for TAT and prothrombin activation peptide (F_{1+2}). **B**, Compared with heparin-treated animals, animals treated with IXai revealed significantly increased levels of both markers. * $P < .05$, by ANOVA.

during CPB, supporting the notion that the surgical wound itself (and thus the extrinsic system) represents a critical stimulus to activation.

Taken together, these data suggest that all 3 predominant stimuli contribute to the enormous prothrombotic stimulus during CPB. We based our studies on the premise that each of these stimuli contribute distinctively to the activation of coagulation and therefore may be inhibited in a targeted manner. We propose that the ideal anticoagulant would selectively block those stimuli that contribute to intravascular coagulation,

Fig 4. Anti-TF IgG or nonimmune (mouse) IgG-stained sections of pericardium (original magnification $\times 25$) revealed striking TF expression (**A**) in pericardial macrophages, (**B**) in the vasculature that pervade pericardial tissue, or (**C**) in pericardial mesothelial cells; (**D**) staining of an adjacent section with nonimmune IgG was negative.

while allowing those stimuli responsible for extravascular hemostasis to proceed unimpeded.

Heparin, the traditional anticoagulant used in CPB, blocks both the intrinsic and extrinsic pathways at multiple sites in the cascade.⁹⁻¹² In contrast, factor IX occupies a unique position in the coagulation cascade and therefore may be a perfect target for a more selective anticoagulant strategy (Fig 1). Blockade of factor IX/IXa inhibits the progression of the classic intrinsic pathway by impairing thrombin generation from blood contact with the bypass circuit. Furthermore, in the setting of low levels of TF (such as those expressed on activated monocytes), inhibition of factor IX/IXa will also block activation of factor X.⁹⁻¹³ However, in the presence of high concentrations of TF (such as those present in the surgical wound), inhibition of factor IX will not affect the direct activation of X that will proceed unimpeded via the TF-factor VIIa-mediated pathway.¹⁶

In this report, we demonstrate that IXai functions as

an effective anticoagulant in primate CPB, maintaining blood fluidity in the extracorporeal circulation while preserving hemostasis in the surgical wound. Furthermore, by evaluating the sites and extent of thrombin generation in this model, these data underscore the separate contributions of intrinsic and extrinsic stimuli in the overall procoagulant response to CPB. Significantly increased levels of TAT and F_{1+2} in the peripheral plasma of IXai-treated animals on bypass demonstrate that overall thrombin generation is greater in baboons treated with IXai compared with those treated with heparin. We attribute these findings to the relative preservation of extravascular hemostasis and thrombin generation in the surgical wound of animals treated with IXai because there was no evidence of intravascular thrombosis.

Further, in preliminary studies in an isolated percutaneous model of CPB (data not shown), peripheral thrombin generation (TAT and F_{1+2}) was found to be

equivalent in IXai- and heparin-treated animals. In sternotomy controls without CPB, peripheral thrombin generation was significantly greater in IXai-treated animals compared with those treated with heparin. Because these models isolate the separate contributions of the surgical wound (sternotomy model) and the CPB circuit (percutaneous model), these additional preliminary findings suggest that activation of coagulation within the bypass circuit may be attributed solely to stimulation of the intrinsic pathway with a contribution from TF expressed on adherent monocytes. Consequently, activation in the surgical wound may be separately attributable to stimulation of the extrinsic pathway.

Moreover, in support of the notion that activation of the extrinsic pathway occurs uniquely in the surgical wound, in this study we demonstrated monocyte TF activity to be different in the intravascular as compared with the extravascular space in IXai-treated animals. Retrieval of monocytes in both IXai- and heparin-treated animals from the intravascular space (peripheral blood, the arterial filter, and the CPB circuit) revealed no demonstrable TF activity. In contrast, only in IXai-treated animals did monocytes isolated from the extravascular space (pericardial blood) manifest significantly enhanced TF activity. Such results are consistent with those of Chung and colleagues,⁸ who demonstrated similar findings of increased pericardial monocyte TF activity.⁸ Interestingly, our data may also help to explain the more recent findings of Ernofsson and colleagues,²⁹ who described no TF expression by circulating peripheral blood mononuclear cells during CPB.

Complementing these findings of increased TF activity in the surgical wound, results of our immunohistochemical examination of the pericardium during CPB demonstrated cellular TF expression. Our findings of striking TF expression by pericardial mesothelial cells, and by the vasculature and macrophages that pervade pericardial tissue, further localize TF to the surgical wound where its presence may lead to direct activation of the extrinsic system.³⁰

Although multiple studies have implicated an inevitable contribution from the intrinsic system during CPB, our data support additional roles for both activated monocytes and for the extrinsic system. Thus despite the complex set of intrinsic/extrinsic interactions that together underlie the coagulopathy of CPB, targeted selective anticoagulation not only represents a feasible antithrombotic strategy but also unveils an ideal anticoagulant approach for cardiac surgery. During CPB, selective anticoagulation with IXai takes advantage of the described distinct procoagulant stimuli and

thereby allows for effective protection against intravascular/low TF-mediated thrombosis, while protecting surgical hemostasis.

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REFERENCES

1. Ovrum E, Brosstad Holen AM, Tangen G, Abdelnoor M. Effects on coagulation and cardiopulmonary bypass. *Circulation* 1995;92:2579-84.
2. Edmunds LH Jr. Blood-surface interactions during cardiopulmonary bypass. *J Card Surg* 1993;8:404-10.
3. Tanaka K, Takao M, Yada I, Yuasa H, Kusagawa M, Deguchi K. Alterations in coagulation and fibrinolysis associated with cardiopulmonary bypass during open heart surgery. *J Cardiothoracic Anaesth* 1989;3:181-8.
4. Colman RW. Hemostatic complications of cardiopulmonary bypass. *Am J Hematol* 1995;48:267-72.
5. Burman JF, Chung HI, Lan DA, Philippou H, Adami A, Lincoln JCR. Role of factor XII in thrombin generation and fibrinolysis during cardiopulmonary bypass. *Lancet* 1994;344:1192-3.
6. Bosclaire MD, Lane DA, Philippou H, Scheikh S, Hunt B. Thrombin production, inactivation and expression during open heart surgery measured by assays for activation fragments including a new ELISA for prothrombin fragment F1+2. *Thromb Hemost* 1993;70:253-8.
7. Kappelmayer J, Bernabei A, Edmunds LH Jr, Edgington TS, Colman RW. Tissue factor is expressed on monocytes during stimulated extracorporeal circulation. *Circ Res* 1993;72:1075-81.
8. Chung JH, Gikakis N, Rao AK, Drake TA, Colman RW, Edmunds LH. Pericardial blood activates the extrinsic coagulation pathway during clinical cardiopulmonary bypass. *Circulation* 1996;93:2014-8.
9. Brister SJ, Ofofu FA, Buchanan MR. Thrombin generation during cardiac surgery: heparin the ideal anticoagulant. *Thromb Hemost* 1993;70:259-62.
10. Khuri SD, Valeri CR, Loscalzo J, Weinstein MJ, Birjiniuk V, Healey NA, et al. Heparin causes platelet dysfunction and induces fibrinolysis before cardiopulmonary bypass. *Ann Thorac Surg* 1995;60:1008-14.
11. Vander Kamp KW, van Deveren W. Contact, coagulation and platelet interaction with heparin treated equipment during heart surgery. *Int J Artif Organs* 1993;16:836-42.
12. Pixley RA, Csello A, DeLa Cadena RA, Kaufman N, Colman RW. Effect of heparin on the activation of factor XII and the contact system in plasma. *Thromb Haemost* 1991;66:540-7.
13. Stern D, Nawroth P, Kiesel W, Vehar G, Esmon C. The binding of factor IXa to cultured bovine endothelial cells: induction of a specific site in the presence of factors VIII and X. *J Biol Chem* 1985;260:6717-22.
14. Thompson A. Structure, function and molecular defects of factor IX. *Blood* 1986;67:565-72.
15. Osterud B, Rapaport SI. Activation of factor IX by the reaction product of tissue factor and factor VII: additional pathway for initiating blood coagulation. *Proc Natl Acad Sci U S A* 1977;74:5260-4.

16. Toomey J, Smith K, Stafford D. Localization of human tissue factor recognition determinants of human factor VIIa. *J Biol Chem* 1991;266:19198-202.
17. Osterud B. Cellular interactions in tissue factor expression by blood monocytes. *Blood Coagul Fibrinolysis* 1995;6(suppl):S20-5.
18. Spanier T, Oz M, Minanov O, Simantov R, Kiesel W, Stern D, et al. Heparinless cardiopulmonary bypass using active site blocked factor IXa: a preliminary study in the dog. *J Thorac Cardiovasc Surg* 1998;115:1179-88.
19. Braunstein KM, Noyes CM, Griffith MJ, Lundblad RL, Roberts HR. Characterization of the defect in activation of factor IX Chapel Hill by human factor XIa. *J Clin Invest* 1981;68:1420-6.
20. Powers JC. Haloketone inhibitors of proteolytic enzymes. In: Weinstein B, editor. *Chemistry and biochemistry of amino acids, peptides and proteins*. vol. 4. New York: Marcel Dekker; 1977. p. 65-178.
21. Lollar P, Fass D. Inhibition of activated porcine factor IX by dansyl-glutamyl-glycyl-arginyl-chloromethylketone. *Arch Biochem Biophys* 1984;233:673-82.
22. Benedict CR, Ryan J, Wolitzky B, Ramos R, Gerlach M, Tijburg P, et al. Active site-blocked factor IXa prevents intravascular thrombus formation in the coronary vasculature without inhibiting extravascular coagulation in a canine thrombosis model. *J Clin Invest* 1991;88:1760-5.
23. Echin P. The scanning electron microscope and its application to research. *Microscopica Acta* 1973;73:189-204.
24. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. *Scan J Clin Lab Invest* 1968;21:77-89.
25. Barstad RM, Ovrum E, Ringdal MA, Oystese R, Hamers MJA, Veiby OP, et al. Induction of monocyte tissue factor procoagulant activity during cardiopulmonary bypass surgery is reduced with heparin coated extracorporeal circuits. *Br J Haematol* 1996;94:517-25.
26. Greogory SA, Edgington TS. Tissue factor induction in human monocytes: two distinct mechanisms displayed by different allo-antigen responsive T cell clones. *J Clin Invest* 1985;76:2440-5.
27. Shigemitsu O, Hadama T, Takasaki H, Mori Y, Kimura T, Miyamoto S, et al. Biocompatibility of a heparin-bonded membrane oxygenator (Carmeda MAXIMA) during the first 90 minutes of cardiopulmonary bypass: clinical comparison with the conventional system. *Artif Organs* 1994;18:936-41.
28. Sundaram S, Courtney JM, Taggart DP, Tweddel AC, Martin W, McQuiston AM, et al. Biocompatibility of cardiopulmonary bypass: influence on blood compatibility of device type, mode of blood flow and duration of application. *Int J Artif Organs* 1994;17:118-28.
29. Ernfors M, Thelin S, Siegbahn A. Monocyte tissue factor expression, cell activation, and thrombin formation during cardiopulmonary bypass: a clinical study. *J Thorac Cardiovasc Surg* 1997;113:576-84.
30. Weiss HJ, Turitto VT, Baumgartner HR, Nemerson Y, Hoffman T. Evidence for the presence of tissue factor activity on subendothelium. *Blood* 1989;73:968-75.

Discussion

Dr Eric A. Rose (*New York, NY*). What happens to the platelets, if anything, in this milieu?

Dr Spanier. We have looked at sensitive markers of platelet, first by studies of platelet aggregation, where we found that there was no evidence of enhanced platelet aggre-

gation in the presence of IXai, whereas there is significantly enhanced platelet aggregation in the presence of heparin. Further, in preliminary studies that we have been able to do recently in an extended primate model of CPB, we found by looking for surface markers of platelet activation (such as CD62, P-selectin, and CD41 and 42) that there is no significant increase in IXai-treated animals whereas there is a significant increase in all of these markers in heparin-treated animals. Thus we believe there is a relative preservation of platelet function in the presence of IXai.

Dr Jack G. Copeland III (*Tucson, Ariz*). I am worried about the elevation of intravascular thrombin in this therapy and the deleterious effects it may have as an inflammatory, a platelet stimulatory, and a prothrombotic agent. What are your comments on that?

Dr Spanier. We recently went to Saudi Arabia where we operated on 88 baboons and were able to do a study that is more comprehensive than this one. In that study we were able to isolate the surgical wound, the CPB circuit, and the entire effect of bypass circuit and surgical wound by doing 3 models of CPB: a percutaneous model, a model in which we only did a median sternotomy, and then a routine, standard open CPB through a median sternotomy.

In that model what we found (which we were hoping to find) was that thrombin generation, although elevated in the open CPB model in the peripheral plasma, was significantly attenuated to the level of the heparin-treated animals in the percutaneous model, further supporting our contention that the stimulus for thrombin generation was in fact in the surgical wound. In the absence of that surgical wound, that stimulus was taken away. Furthermore, the absence of intravascular thrombus suggested to us that the markers of peripheral thrombin generation did not have a significant effect on the procoagulant milieu.

Furthermore, you addressed the issue of inflammatory activation, which is certainly one of the most important in the setting of CPB. By using these 3 separate models, we found that not only do we have attenuated intravascular thrombus with IXai, but more important, perhaps, in the presence of IXai as compared with heparin, we have attenuation of complement, inflammatory mediators, such as interleukin-6 and interleukin-8 and the described decreases in platelet activation. Not only do we think that IXai is going to represent a targeted anticoagulant approach, but it also suggests the critical interactions between the proinflammatory and procoagulant milieu during CPB and further suggests the targeted directed approach to block both the procoagulant and the proinflammatory response to cardiac surgery.

Dr John C. Chen (*Orange, Calif*). With IXai, do you have evidence that this is a specific inhibitor for factor IXa? Is this applicable to human beings? Is IXai that is specific for monkeys applicable to the human species?

Dr Spanier. IXai is specific for the inhibition of factor IX in plasma. We know this by both binding studies. We found in dose-response studies that, at low doses and at the antithrombotic dose that we use of IXai (which is about 460 µg/kg), we have specific factor IX inhibition, which we test

using factor-deficient plasmas. We are able to show that there is no inhibition of factor XII, XI, or VIII during the procedure, whereas there is specific inhibition of IX. At higher doses of IXai, though, we do find that there is some inhibition of the other coagulation factors.

Regarding the second question that you had about the clinical applicability, we have had the fortunate experience at Columbia of using Institutional Review Board exemptions in several patients who had been critically ill and had no real alternative, to be able to use IXai in the clinical setting. We have used it in the setting of CPB and in the setting of other extracorporeal circuits, such as extracorporeal membrane oxygenation (ECMO). We found in those patients that there is significant attenuation of thrombin generation in the peripheral plasma, and we were able to keep those circuits that I have just described open in the setting of IXai with no demonstrable adverse effects to this treatment. Furthermore,

what we found in these patients is that we were able to stop the bleeding extravascularly in their wounds and to anticoagulate their circuits.

Dr Ahmad Rajaii Khorasan (*Neptune, NJ*). Do you think this agent will block the stimulatory effect of heparin on platelets?

Dr Spanier. We have actually done the studies using both heparin and IXai in the same platelet aggregometer, and we found that IXai does seem to attenuate some of the heparin effects on platelets. We are not exactly sure of the mechanism of this yet, unless IXai does truly have a protective effect on platelets. What we think is more likely is that there are critical interactions between the ways that the inflammatory system (as I described) and the coagulation system are activated, and platelets probably play an important role there. IX may be one of the markers, one of the kind of entrances to the critical interactions between the inflammatory and the coagulant pathways.