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SURFACE-BOUND HEPARIN FAILS TO REDUCE THROMBIN FORMATION DURING CLINICAL CARDIOPULMONARY BYPASS

The hypothesis that heparin-coated perfusion circuits reduce thrombin formation and activity; fibrinolysis; and platelet, complement, and neutrophil activation was tested in 20 consecutive, randomized adults who had cardiopulmonary bypass. Twenty identical perfusion systems were used; in 10, all blood-contacting surfaces were coated with partially degraded heparin (Carmeda process; Medtronic Cardiopulmonary, Anaheim, Calif.). All patients received a 300 U/kg dose of heparin. Activated clotting times were maintained longer than 400 seconds. Cardiopulmonary bypass lasted 36 to 244 minutes. Blood samples for platelet count, platelet response to adenosine diphosphate, plasma β-thromboglobulin, inactivated complement 3b, neutrophil elastase, fibrinopeptide A, prothrombin fragment F1.2, thrombin-antithrombin complex, tissue plasminogen activator, plasminogen activator inhibitor-1, plasmin α_2-antiplasmin complex, and D-dimer were obtained at these times: after heparin was given, 5 and 30 minutes

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after cardiopulmonary bypass was started, within 5 minutes after bypass was stopped, and 15 minutes after protamine was given. After cardiopulmonary bypass, tubing segments were analyzed for surface-adsorbed antithrombin, fibrinogen, factor XII, and von Willebrand factor by radioimmunoassay. Heparin-coated circuits significantly ($p < 0.001$) reduced platelet adhesion and maintained platelet sensitivity to adenosine diphosphate ($p = 0.015$), but did not reduce release of β-thromboglobulin. ***There were no significant differences between groups at any time for fibrinopeptide A, prothrombin fragment F1.2, or thrombin-antithrombin complex or in the markers for fibrinolysis: D-dimer, tissue plasminogen activator, plasminogen activator inhibitor-1, and α_2-antiplasmin complex.*** **In both groups, concentrations of prothrombin fragment F1.2 and thrombin-antithrombin complex increased progressively and significantly during cardiopulmonary bypass and after protamine was given. Concentrations of D-dimer, α_2-antiplasmin complex, and plasminogen activator inhibitor-1 also increased significantly during bypass in both groups. Fibrinopeptide A levels did not increase during bypass but in both groups increased significantly after protamine was given. No significant differences were observed between groups for levels of inactivated complement 3b or neutrophil elastase. Radioimmunoassay showed a significant increase in surface-adsorbed antithrombin on coated circuits but no significant differences between groups for other proteins. We conclude that heparin-coated circuits used with standard doses of systemic heparin reduce platelet adhesion and improve platelet function but do not produce a meaningful anticoagulant effect during clinical cardiopulmonary bypass. The data do not support the practice of reducing systemic heparin doses during cardiac operations with heparin-coated extracorporeal perfusion circuitry. (J THORAC CARDIOVASC SURG 1996;111:1-12)**

Cardiopulmonary bypass (CPB) produces a massive thrombotic stimulus that is only partially suppressed by doses of heparin (3 mg/kg) that are two to three times those used to treat other thrombotic diseases. Despite activated clotting times of more than 400 seconds, thrombin is produced and increases progressively during CPB in every patient.[1, 2] Thrombin is a powerful protease[3] that primarily cleaves fibrinogen to fibrin, directly activates platelets by means of a specific thrombin receptor,[4] and stimulates endothelial cells to produce tissue plasminogen activator.[5] Formation of thrombin is directly related to production of fibrin, platelet dysfunction, and fibrinolysis associated with CPB.

Over the past decade, numerous studies have suggested that heparin-coated extracorporeal perfusion circuits are "more biocompatible" (i.e., activate blood elements less strongly) than uncoated systems.[6-12] Some of these studies indicate that heparin-coated circuits reduce complement activation,[6-9] reduce platelet[8, 11] and neutrophil[7] activation, and reduce the need for standard doses of heparin.[9, 12, 13] The efficacy of heparin-coated surfaces with respect to complement, platelet, and leukocyte activation relates to the "inflammatory response" and morbidity of CPB. However, if standard doses of systemic heparin are reduced, the anticoagulant efficacy of heparin-coated perfusion circuits relates directly to the safety and mortality of CPB.

This study was designed to determine the effect of heparin-coated perfusion circuitry on thrombin production and activity; fibrinolysis; platelet, neutrophil, and complement activation; and surface adsorption of selected plasma proteins during clinical cardiac operations in adults. Because thrombin is formed during CPB even with standard doses of systemic heparin, we tested the hypothesis that use of heparin-coated perfusion circuitry reduces thrombin formation and activity during CPB and has an anticoagulant effect.

Methods

Over a 2-month period, 20 consecutive (with one exception), consenting patients who had cardiac operations during regular weekday hours were randomized by card

drawing into two groups—a control group in which the perfusion circuitry *was not* coated with heparin and a group in which the perfusion circuitry *was* coated with heparin. The study was approved by the Human Research Committee of the University of Pennsylvania. The excluded patient had postinfarction angina, two previous operations, a preoperative intraaortic balloon pump, and mechanical circulatory assistance after revascularization. No other patient was excluded for any reason. All patients whose blood samples could be processed during regular weekday hours were asked to join the study and none refused.

Twenty identical sterile perfusion circuits were assembled consisting of a soft-shell venous reservoir (model MVR1600, Medtronic Cardiopulmonary, Anaheim, Calif.), a centrifugal blood pump (Bio Pump model BP-80, Medtronic Bio-Medicus, Eden Prairie, Minn.), a hollow-fiber membrane oxygenator (Maxima model 1380, Medtronic Cardiopulmonary), and a 40 μm arterial filter (Intersept model M40, Medtronic Cardiopulmonary). The pump and table packs of medical-grade polyvinylchloride tubing (Medtronic Cardiopulmonary) included two lines for suction and one line for actively venting the left side of the heart (model 14622, Sarns 3M Health Care, Ann Arbor, Mich.) with blood returning to a filtered cardiotomy reservoir (Intersept model 1351, Medtronic Cardiopulmonary). For all patients a 4:1 blood cardioplegia system (model 5328, Gish Biomedical, Irvine, Calif.) was used to administer cardioplegic solution, both antegradely through the aortic root and retrogradely through the coronary sinus (cannula model 94215, DLP, Grand Rapids, Mich.). The aorta was cannulated with a 24F aortic cannula (model 4335, Sarns 3M Health Care, Ann Arbor, Mich.), and venous return was achieved with a two-stage venous catheter (model TR3651, Research Medical, Midvale, Utah) in all but four patients. These latter patients required bicaval cannulation (Research Medical).

In 10 perfusion circuits, all components that contacted blood were completely coated with the heparin-bonded Carmeda BioActive Surface (Medtronic Cardiopulmonary). The heparin coating included all cannulas, reservoirs, tubing, connectors, and components of the system; only the Cell Saver System (Haemonetics, Braintree, Mass.) was not coated (explained later). Because the packaged retrograde cardioplegia catheter (model 94215, DLP, Grand Rapids, Mich.) cannot be coated, a different catheter (model RC 2012 MIB, Research Medical), which was coated by Medtronic Cardiopulmonary, was used with the coated circuits. The Carmeda process covalently binds partially degraded heparin to aminated biomaterial surfaces by reduction with sodium cyanoborohydride.[14] Control perfusion circuits appeared identical and the operating surgeon did not know whether a heparin-coated circuit was used until the retrograde cardioplegia catheter was inserted.

In all patients, intraoperative autotransfusion was used to collect and process shed blood from the operative field with the use of an uncoated autologous Cell Saver System (model 243, Haemonetics) composed of two suction lines and an unfiltered cardiotomy reservoir (model MCR4000, Medtronic Cardiopulmonary). Each unit of packed red blood cells salvaged through this system was washed with 1 L of normal saline solution before filtered reinfusion.

All patients received isoflurane (Forane) anesthesia and vancomycin and cefazolin. Anesthesia was induced with midazolam in seven patients and with fentanyl in 13. Eighteen had pancuronium bromide. All except one had Swan-Ganz catheters (Baxter Healthcare Corp, Edwards Division, Santa Ana, Calif.), and all had arterial catheters. After anesthesia, intubation, and midline sternotomy, all patients received an intravenous dose of bovine lung heparin, 300 U/kg, before cannulation. Activated clotting times were measured (Hemochron model 801, International Technidyne Corp., Edison, N.J.) at baseline, after administration of heparin before the start of CPB, and every 30 minutes during CPB. If necessary, additional boluses of heparin were given to maintain clotting times longer than 400 seconds throughout the period of CPB. The left ventricle was vented from the right superior pulmonary vein–left atrial junction in all patients except those with bicaval cannulation.

Sixteen patients were cooled to a mean nasopharyngeal temperature of 30.6° ± 0.7° C (standard error). The four patients who had pulmonary thromboembolectomy were cooled to 12° to 14.9° C and each had four or five periods of circulatory arrest for 11 to 14 minutes that totaled 46 to 69 minutes (mean 54.5 ± 5 minutes). After aortic clamping all hearts were arrested with intermittent, cold blood, potassium cardioplegic solution administered in an antegrade fashion; alternating retrograde cardioplegia was used in all except the patients who required thromboembolectomy and one patient with an atrial septal defect. Myocardial temperature was monitored and kept between 8° and 14° C. Additional surface cooling with cold saline solution was used to maintain myocardial hypothermia. All field blood was returned either directly to the perfusion system or to the Cell Saver System (Haemonetics).

Blood samples (20 ml) were obtained from the arterial catheter 5 minutes after heparin was administered, 5 and 30 minutes after the start of CPB, approximately 5 minutes before CPB was stopped, and 15 minutes after protamine was given. After wound closure, chest tube drainage was measured hourly for 24 hours and returned to the patient through an autotransfusion system (Pleurevac model A-7050-ATS, Deknatel, Inc., Fall River, Mass.).

In 12 patients (six from each group), 5 to 7 cm lengths of both venous and arterial tubing were excised from the perfusion circuit immediately after the operation and preserved in 2.5% buffered glutaraldehyde solution. The presence of four surface-adsorbed proteins—antithrombin, fibrinogen, factor XII, and von Willebrand factor—was detected by radioimmunoassay (RIA)[15] in all samples.

Whole blood platelet counts were obtained in duplicate with a hemocytometer by means of phase microscopy. Platelet aggregation was measured in response to adenosine diphosphate in platelet-rich plasma.[16] The number of platelets in platelet-rich plasma was normalized to 150,000/μL in all samples to standardize aggregation. Threshold doses of adenosine diphosphate were determined as the lowest dose of agent able to produce biphasic aggregation of 62.5% or more after 5 minutes in control

Table I. *Patient descriptors and operations*

Descriptor	*Control (n = 10)*	*Heparin surface (n = 10)*
Age (yr) (mean ± SEM)	64.8 ± 4.1	61.0 ± 3.5
Male/female	5/5	6/4
Operation		
Myocardial revascularization		
First operation	4	4
Second operation	1	
Valve repair/replacement		
Aortic		3
Mitral	1	1
Both	1	
Pulmonary thromboembolectomy	2	1
With CABG ×3		1
Closure of atrial secundum defect	1	
Aortic crossclamp time (min)	81.0 ± 14.8	80.0 ± 12.4
Duration of CPB (min)	121 ± 20.0*	115.4 ± 15.7*
Mean ± SEM		
Range (min)	36-211	71-216

CABG, Coronary artery bypass grafting; *SEM,* standard error of the mean.
*Includes periods of circulatory arrest.

Table II. *Relevant drugs, blood losses, and transfusions*

	Control (n = 10)	*Coated (n = 10)*
Total heparin (units in OR)	23,250 ± 3,374	32,150 ± 3,106
Total protamine (mg or OR)	134 ± 13.3	183 ± 15.5*
Chest tube drainage (ml in 24 hr)	555 ± 83†	780 ± 213
Mean ± SEM		
Range (ml)	175 – 1025	312 – 2620
Blood product transfusions (units in hospital)		
Packed cells	24†	25
Fresh frozen plasma	5†	13
Platelet units	6†	24

OR, Operating room; *SEM,* standard error of the mean.
*$p < 0.05$.
†Excludes data from one patient.

samples. Threshold doses of adenosine diphosphate were then used to measure aggregation as a function of percent light transmittance in subsequent samples. An RIA was used to measure plasma levels of platelet β-thromboglobulin.[17] Plasma neutrophil elastase[18] and inactivated complement 3b,[19] a marker for complement activation, were measured by means of indirect competitive enzyme-linked immunosorbent assays (Quidel Inc., San Diego, Calif., for inactivated complement 3b). Enzyme-linked immunosorbent assays for fibrinopeptide A (American Bioproducts, Parsipanny, N.J.),[20] D-dimer (American Diagnostica Inc., Greenwich, Conn.),[21] F1.2, a marker of prothrombin cleavage (Behring Diagnostics Inc, Westwood, Mass.),[22] and thrombin-antithrombin complex (Behring Diagnostics Inc.)[23] were performed according to instructions. Tissue plasminogen activator[24] and plasminogen activator inhibitor-1[25] were measured by enzyme-linked immonoassay kits from American Diagnostica Inc.; plasmin α_2-antiplasmin was also measured by enzyme-linked immunoassay (Behring Diagnostics Inc.).[26]

For RIAs of adsorbed proteins, tubing samples were cut into 1 cm length rings and bisected twice into quarter segments. Each quarter segment had an inner surface area of 0.8 cm^2. Duplicate tubing samples were placed in glass tubes for RIA of each adsorbed protein. Samples were rinsed twice with phosphate-buffered saline solution (PBS) (1000-3, Sigma Chemical Company, St. Louis, Mo.) followed by addition of 2 ml of milk solution (15% nonfat dry milk in PBS with a 2 mmol/L concentration of sodium ethylenediaminetetraacetic acid). Samples were refrigerated overnight and rinsed three times with PBS. Primary antibodies and rabbit antihuman immunoglobulin G fractions (10 mg/ml stock) were diluted 1:500 in PBS[16] containing 1% chicken egg albumin (ovalbumin), and 1 ml was added to each sample for 60 minutes at 37° C. Samples were again rinsed three times with PBS and the milk solution was added for storage overnight at 4° C. After each sample was rinsed three times with PBS, 1 ml of ^{125}I-labeled goat antirabbit immunoglobulin G (7 μCi/ml, 250,000 cpm) was added to each sample for 60 minutes at 37° C. After incubation, samples were rinsed three times with PBS, placed into a clean test tube, and counted in a gamma counter.[15] Counts of ^{125}I-labeled goat antirabbit immunoglobulin G bound to the primary antibody of each target protein and a control count (1% ovalbumin/PBS) were recorded. Control counts were subtracted from counts recorded for each target protein.

Data are expressed as mean and standard error of the mean. Comparisons to "After heparin, before CPB" at different time intervals within groups were made by means of the paired *t* statistic. Comparisons between groups were made with the use of the *t* statistic for independent samples with the Bonferroni correction for multiple comparisons (SYSTAT for Windows 5, SYSTAT, Inc., Evanston, Ill.) and by analysis of variance for repeated measures (SPSS for Windows 6.1, SPSS, Inc., Chicago, Ill.). The *t* statistic shows significant differences between groups at specific time points; the analysis of variance compares the differences between the two groups for all samples obtained after CPB began. A *p* value less than 0.05 is considered significant.

Results

Descriptive data for the 20 patients appear in Table I. There were no significant differences between groups in the demographic data. Three patients (one from the control group; two from the coated group) had aspirin within 5 days of the operation. Six patients (four control) received heparin intravenously or subcutaneously and one control patient received warfarin sodium (Coumadin) before the operation.

Twenty-four–hour postoperative blood loss, heparin and protamine doses, and autologous blood and

Table III. *Blood and plasma concentrations (means ± standard error)*

	After heparin, before CPB		*5 min CPB*		*30 min CPB*		*After CPB*		*After protamine*		
Measurement	*Control*	*Coated*	*Control*	*Coated*	*Control*	*Coated*	*Control*	*Coated*	*Control*	*Coated*	*p**
Platelet count	98.5	106.5†	76.2§	106.5†	69.6§	102.8	78.5‡	101.4	78.8‡	91.4	0.001
(% of initial count)	±2.1	±3.2	±3.1	±7.2	±6.1	±11.3	±8.5	±13.0	±7.6	±10.5	
Platelet aggregation	102.7	106.9	88.2§	101.6	79.9§	98.0	74.2‡	89.3‡	57.1§	75.4‡	0.015
(% light transmittance)	±5.0	±6.1	±5.0	±5.6	±8.6	±8.0	±9.8	±15.0	±8.3	±10.0	
Plasma β-thromboglobulin	9.3	10.1	15.2‡	11.6	21.2§	13.6‡	214§	226.1§	198.3§	220.3§	0.789
(μg/ml)	±1.0	±0.7	±1.9	±1.4	±2.4	±1.3	±32.1	±40.5	±36.4	±11.5	
F1.2 (nm)	2.3	2.3	3.4§	3.1§	3.8§	3.4§	5.5§	5.7§	5.6§	6.2§	0.965
	±0.15	±0.18	±0.39	±0.25	±0.40	±0.35	±0.60	±0.76	±0.54	±0.75	
FPA (ng/ml)	12.4	8.5	7.6‡	6.9	4.9§	2.4‡	8.7	6.2	30.2§	28.71§	0.378
	±2.2	±2.6	±1.5	±2.7	1.5	±0.6	±2.7	±1.7	±4.8	±4.4	
TAT (μg/L)	14.6	12.1	37.0§	27.9§	39.6§	27.5§	51.3§	51.6	51.1§	54.4§	0.169
	±1.8	±1.7	±5.9	±4.0	±5.3	±3.3	±3.9	±4.8	±4.2	±4.0	
D-dimer (mg/L)	0.26	0.26	0.36	0.26	0.43	0.28	0.82‡	0.65§	0.66‡	0.66§	0.265
	±0.04	±0.03	±0.11	±0.03	±0.13	±0.03	±0.23	±0.11	±0.18	±0.08	
t-PA (ng/ml)	11.7	7.6	9.4	6.6	12.5	8.8	12.4	12.2§	14.5‡	14.1§	0.176
	±2.1	±0.8	±1.8	±0.5	±2.6	±0.4	±1.9	±1.0	±1.5	±1.2	
PAI-1 (ng/ml)	36.3	34.7	28.9	26.4‡	40.4	33.4	48.9§	48.6§	49.4§	57.2§	0.877
	±5.3	±5.4	±3.9	±3.7	±4.5	±5.0	±4.9	±4.8	±5.6	±3.1	
APP (ng/ml)	884	696	852	593	1712	822	2018§	1502§	1998§	1632§	0.013
	±166	±63	±132	±75	±561	±136	±330	±210	±317	±211	
Elastase (ng/ml)	67.2	75.0	64.0	53.0	88.1‡	71.0	127.6§	96.4	139.2§	120.0‡	0.533
	±11.5	±15.4	±10.3	±9.6	±6.0	±12.4	±11.1	±23.8	±15.3	±24.8	
iC3b (μg/ml)	33.8	47.4	15.8‡	27.1	18.2‡	36.8	17.6	24.9	30.5	34.8	0.118
	±5.8	±11.8	±2.1	±7.1	±1.6	±12.1	±1.6	±2.4	±3.5	±5.1	
Hematocrit value (%)	32.7	35.6	21.5	24.4	21.6	25.1	24.0	23.7	23.6	25.1	
	±1.8	±2.5	±1.4	±1.6	±1.6	±1.7	±1.3	±1.6	±1.0	±1.7	

F1.2, Prothrombin fragment F1.2; *FPA,* fibrinogen peptide A; *TAT,* thrombin-antithrombin complex; *D-dimer,* fibrin fragment, D-dimer; *Elastase,* neutrophil elastase; *iC3b,* inactivated C3b; *t-PA,* tissue plasminogen activator; *PAI-1,* plasminogen activator inhibitor-1, *APP,* plasmin α_2-antiplasmin complex.

*p Value for F statistic by analysis of variance for repeated measures. Baseline date (after heparin, before CPB) were excluded.

†$P < 0.01$ ± standard error, between-groups t statistic with Bonferroni correction.

‡$p < 0.05$ ± standard error, within groups, compared with "after heparin, before CPB."

§$p < 0.01$ ± standard error, within groups, compared with "after heparin, before CPB."

blood product transfusions during hospitalization appear in Table II. One 75-year-old patient who had pulmonary thromboembolectomy (control group) had excessive nonsurgical bleeding and was returned to the operating room for wound exploration. This patient's 24-hour blood loss was 5875 ml. He received 21 units of packed cells, 29 units of fresh frozen plasma, 54 units of platelets, and 20 units of cryoprecipitate. His blood losses and transfusion history are excluded from Table II. With this exclusion there were no significant differences between the two groups in heparin doses, blood loss, or total blood product transfusions, but patients in whom coated circuits were used required more protamine ($p < 0.05$). Two of nine patients had fresh frozen plasma and one had platelets in the control group; three of 10 had fresh frozen plasma and two had platelets in the heparin-coated group. Nineteen patients were discharged home. The patient with excessive bleeding died of respiratory failure on postoperative day 49.

Changes in measured blood and plasma constituents before, during, and after CPB appear in Table III and in Figs. 1 and 2. Platelet counts are corrected for hemodilution; values for other samples that are produced during CPB are not corrected, but mean hematocrit value is given to enable "corrective" calculations to be made. In patients in whom heparin-coated circuits were used, the corrected platelet count is significantly higher 5 minutes after the start of CPB than in patients in whom uncoated systems were used (Fig. 1). Differences between groups do not reach significance after the Bonferroni correction at other time points, but by analysis of variance the two groups differ significantly ($p = 0.001$). The number of circulating platelets decreases significantly from before CPB in the control group of patients at all subsequent time points and does not

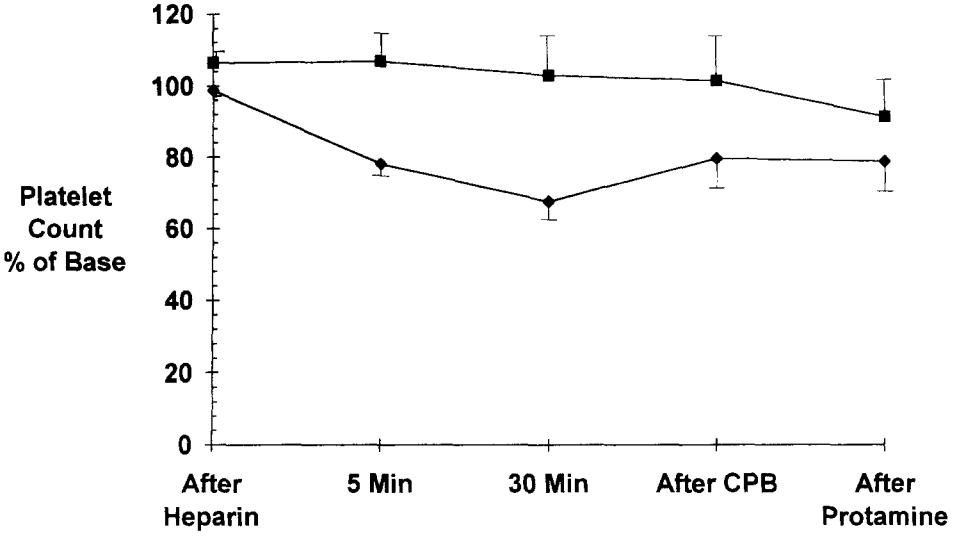

Fig. 1. Platelet counts depicted as percentage of platelet count obtained before heparin at each time interval for each group. Means ± standard error. *Diamonds* indicate control group; *squares* indicate group in which coated circuits were used. See Table III for significant differences within and between groups.

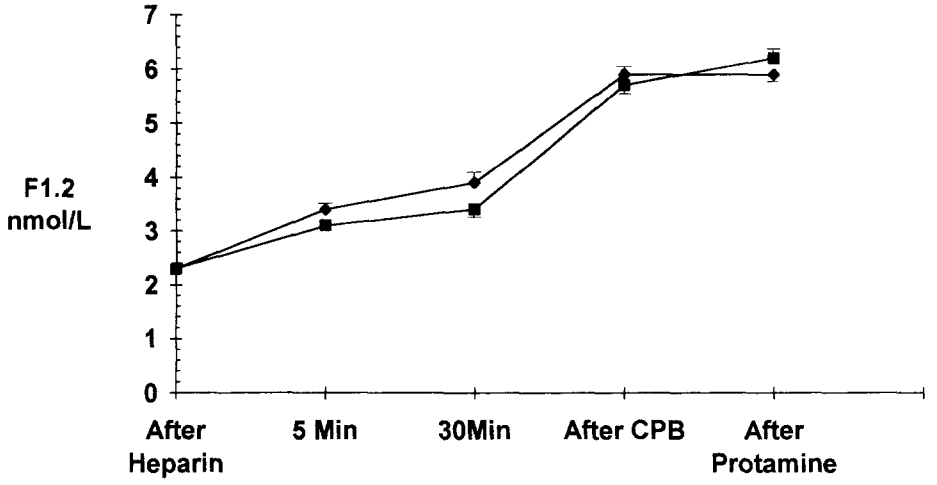

Fig. 2. Plasma prothrombin fragment F1.2 at each time interval. Means ± standard error. *Diamonds* indicate control group; *squares* indicate group in which coated circuits were used. There were no significant differences between groups.

decrease significantly in patients in whom coated systems were used. Platelet responsiveness to adenosine diphosphate is better preserved in patients in whom coated systems were used, and the difference between the two groups is significant by analysis of variance ($p = 0.015$). In both groups plasma β-thromboglobulin concentration increases during CPB, but there are no significant differences between groups at any time nor between the two groups by analysis of variance ($p = 0.789$).

As compared with "After heparin, before CPB," levels of F1.2, thrombin-antithrombin complex, D-dimer, and plasmin α_2-antiplasmin complex are significantly higher at the end of CPB in both groups (Figs. 2 and 3 and Table III). Levels of all of these markers of thrombin formation and activity and fibrinolytic activity, in addition to fibrinopeptide A and tissue plasminogen activator, are significantly higher after protamine in both groups. *There are no significant differences between groups for any of these markers at any time point.* Further, there are no significant differences between groups for markers

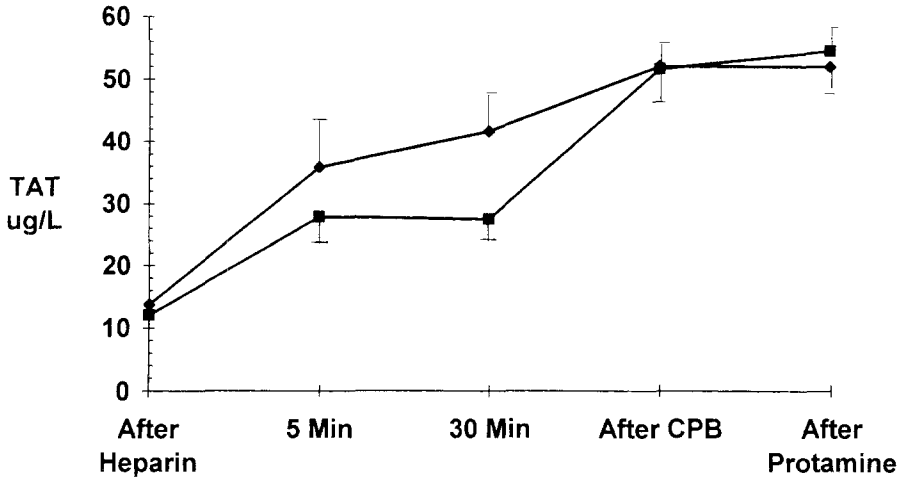

Fig. 3. Plasma thrombin-antithrombin complex *(TAT)* at each time interval. Means ± standard error. *Diamonds* indicate control group; *squares* indicate group in which coated circuits were used. There were no significant differences between groups.

of thrombin formation by analysis of variance (Table III). The concentration of neutrophil elastase increases by the end of CPB in both groups; inactivated complement 3b concentration does not increase during CPB and actually decreases early after CPB starts in all patients. With the exception of plasmin α_2-antiplasmin complex, there are no significant differences between groups by either the *t* statistic or analysis of variance.

Results of RIA measurements of surface-adsorbed proteins appear in Fig. 4. Significantly more antithrombin is adsorbed onto heparin-coated surfaces than onto uncoated surfaces. There are no significant differences between the amounts of other surface-adsorbed proteins between groups and no significant differences for any adsorbed protein between venous and arterial tubing samples.

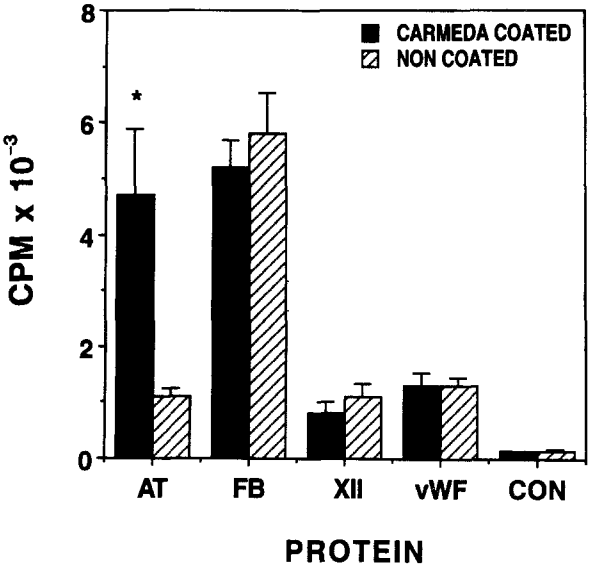

Fig. 4. RIA for detection of adsorbed proteins on surfaces of arterial and venous tubing (n = 24). Data are expressed as counts per minute x 10^3 ± standard error of the mean of secondary ^{125}I- labeled goat antirabbit immunoglobulin G binding to primary antibodies (rabbit anti-human immunoglobulin G) to antithrombin *(AT)*, fibrinogen *(FB)*, factor XII *(XII)*, von Willebrand factor *(vWF)*, or PBS control *(CON)*. $^*p < 0.05$ by Student's unpaired *t* statistic between groups.

Discussion

This study fails to show that heparin-coated extracorporeal perfusion circuits reduce any plasma markers of thrombin formation and activity or any markers of fibrinolytic activity during cardiac operations; therefore we conclude that surface-bound heparin does not have an additional anticoagulant effect greater than that produced by standard doses of systemic heparin on soluble coagulation proteins in vivo. These data and this conclusion confirm and extend the findings of Korn and colleagues[11] and Wagner and coworkers,[27] who also found no evidence of anticoagulant activity of surface-bound heparin in vitro or in vivo. Wagner's group studied patients with first-time isolated myocardial revascularization operations and used perfusion circuits that lacked the heparin coating on the cardiotomy reservoir system; however, their data, taken at more

frequent sampling times, are similar to these with completely heparinized circuits. Although all three studies (Korn's, Wagner's, and this one) used the Carmeda process, the findings are unlikely to differ with ionic bound heparin coatings except for heparin leaching from the latter circuits.[10, 28] In vitro, the competing Duraflo II heparin coating (Bentley Laboratories Division, Irvine, Calif.) leaches approximately 15% of the surface-bound heparin into saline solution within 4 hours.[28] Because surface-bound heparin has no demonstrable additional anticoagulant effect, we believe that reduction of systemic heparin[9, 12, 13] is unwise and may risk thrombosis and embolism during the operation.[29]

In vitro studies show that covalently bound surface heparin does bind antithrombin and catalyzes antithrombin binding to factor Xa[30] and to thrombin.[31] Furthermore, thrombin binds to surface-adsorbed antithrombin[32] and may bind to surface-adsorbed heparin. Surface-adsorbed thrombin does not block the ability of surface-bound heparin to bind more antithrombin when more antithrombin is introduced into the system.[31] These data are consistent with the increased surface antithrombin measured by RIA on the coated circuits in the current study. Normally, heparin catalyzes the binding of antithrombin to thrombin and is not consumed in the process. The in vitro studies[30, 31] indicate that coated surfaces bind thrombin, but it is not clear[27, 32, 33] that the covalently bonded thrombin-antithrombin complex is released by bound heparin. If surface heparin releases thrombin-antithrombin complex, we expect to see higher concentrations of plasma thrombin-antithrombin complex associated with the heparin-coated circuits because thrombin is progressively produced during CPB. No study (references 27, 32, 33, and this study) has shown higher plasma concentrations of thrombin-antithrombin complex at any time during CPB. This study shows nonsignificantly reduced plasma concentrations of thrombin-antithrombin complex during the initial period of CPB, but nearly identical concentrations at the end of CPB. Gu,[32] Pradhan,[33] and their colleagues found reduced plasma thrombin-antithrombin complex throughout CPB, but these investigators did not measure markers of thrombin formation. The absence of significant differences in plasma F1.2 and thrombin-antithrombin complex concentrations in this and Wagner's study[27] are most consistent with the conclusion that surface-bound heparin does not produce an additional anticoagulant effect greater than that produced by standard doses of systemic heparin during clinical CPB.

Subsequent prospective, randomized, controlled studies in patients with coated perfusion circuits and *reduced* levels of systemic heparin may or may not show reduction in both plasma F1.2 and thrombin-antithrombin complex concentrations during CPB; if reductions of both markers occur, the conclusion that coated surfaces produce an anticoagulant effect is justified. However, to dispel the concerns that thrombin-antithrombin complex may not be released from the coated surface and that the coated surface may become saturated with thrombin-antithrombin complex and unable to bind additional thrombin, both markers must remain significantly less than plasma concentrations in control subjects treated with a standard dose of heparin and an uncoated circuit for the full duration of CPB.

Surface-bound heparin alters the composition of surface-adsorbed proteins, changes some characteristics of the resulting protein mosaic, and is not completely buried by adsorbed plasma proteins.[34] As mentioned earlier, we find more antithrombin on coated surfaces by RIA. We also find significantly higher platelet counts and greater platelet sensitivity to adenosine diphosphate during CPB in patients in whom coated circuits were used. Wagner and colleagues[27] find nonsignificantly less β-thromboglobulin release with coated perfusion circuits. These observations are consistent with those of in vitro studies,[11, 35] but other investigators[6, 12, 27] do not observe reduced platelet adhesiveness with clinical applications of heparin-coated surfaces. Thus the effect of heparin coatings on platelets remains unsettled.

Some investigators find less activation of neutrophils[7, 36-38]; others,[33] including us, do not. Three groups report reductions in markers of complement activation at various and often different sampling times during and immediately after cardiac operations.[6, 7, 9, 38] These data suggest that surface-bound heparin may alter the reactivity of some blood elements with circuit surfaces in vivo and may possibly reduce mediators[39] of the "whole body" inflammatory response.[40] However, the magnitude, significance, and importance of these effects, which are not related to coagulation, remain unclear.

In the practice of cardiothoracic surgery, special circumstances occasionally arise wherein administration of systemic heparin is or may be dangerous because of bleeding risks that are remote from the surgical field. Under these circumstances, surgeons

may choose not to use systemic heparin or to reduce the dose as the lesser of competing risks. Although this option is not supported by this and Wagner's study,[27] there are anecdotal reports of successful use of heparin-coated circuits in most applications,[41-44] but not all.[29]

The mechanisms by which thrombin is formed during cardiac operations are still being studied and argued. There is evidence that both the intrinsic[45-46a] and extrinsic[47-48a] coagulation pathways are involved; the relative importance of each is disputed. There is also substantial evidence that heparin is an agonist for platelets,[49, 50] complement activation,[51] and neutrophils.[45, 52] Putting these issues aside, there is universal agreement that cardiac operations produce a massive thrombotic stimulus[1, 2] that must be suppressed by high concentrations of heparin. Whether surface-bound heparin circuits are more thromboresistant than uncoated circuits or whether they reduce the "whole body inflammatory response" is irrelevant if coated surfaces do not produce a meaningful and reliable anticoagulant effect in vivo when doses of systemic heparin are reduced.

After presentation before The American Association for Thoracic Surgery and in peer review, this study was criticized for small sample size and lack of statistical power. It should be pointed out that the biochemical assays and surface protein maps used in this study are both sophisticated and expensive, and they address the issue of thrombin formation with the most powerful methods currently available. Statistical power is influenced by both sample size and the magnitude of the differences between means. When observed differences are large (e.g., platelet counts in this study), the power value is large (e.g., 0.986 for these platelet counts) even though the sample size is small. Power is primarily used to design studies to determine how many samples are needed to prove or disprove *a chosen* difference between groups, which the investigator arbitrarily decides is meaningful and important. Our group could not decide what differences to choose, because CPB alters all of the parameters studied and all of the alterations are undesirable.

This argument, however, misses the point and purpose of this study. Our group became concerned that perfusion was being done with coated circuits and half the standard dose of heparin, despite the absence of prior, reliable measurements of thrombin formation. The most important point that the study makes is that *the efficacy of heparin-coated circuitry to reduce thrombin formation and activity during CPB has not been demonstrated.* The burden of proof in science is to demonstrate a phenomenon, in this case that heparin coatings reduce thrombin formation and activity. The null hypothesis is accepted until it is shown with high probability to be false. No scientist has ever proved that a phenomenon does not happen. As a reviewer noted, "absence of proof is not proof of absence." We agree, but we hope that cardiac surgeons will not abandon science for theology.

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Discussion

Dr. Shukri F. Khuri *(West Roxbury, Mass.).* The strength of this work is that it directs to an unsettled field of investigation a host of much needed state-of-the-art hematologic assays, providing us with a compendium of end points that should enhance our understanding of the prosthetic surfaces–blood interface.

My main concern about this study relates to the relatively small sample size, 10 patients in each group, and its impact on the interpretation of the data. The study concluded that heparin-coated circuits did not offer an advantage over conventional circuits mainly because, in the course of CPB, they did not effect a significant reduction in the degree of thrombin formation (as assessed by thrombin-antithrombin complex, fibrinopeptide A, and prothrombin fragment F1.2) or fibrinolysis (assessed with D-dimer, tissue plasminogen activator, plasminogen activator inhibitor-1, and plasmin α_2-antiplasmin complex. The authors did not provide power statistics to ensure against type II statistical errors. A close examination of Table III shows that trends *in favor of the heparin-coated surfaces* were observed in all but one of these parameters at the 30-minute CPB time point. Considering the relatively small size of the two patient groups, how can the authors assure us that the lack of statistical significance that they attributed to these trends was not a reflection of a type II statistical error?

I also have concerns about the lack of correction of the concentration of the various proteins for the significant amount of hemodilution that must have occurred with the institution of CPB. Systemic hypothermia, the extent of which varied widely in these patients, was also not shown to be comparable in the two groups. Data from our institution lead us to hypothesize that the salutary hematologic effects of heparin-coated circuits are best realized under normothermic conditions. Could the wide range of systemic hypothermia encountered in this study have blunted the salutary effect of the heparin-coated circuit?

Last, considering that heparin per se, independent of CPB, has been shown to cause significant platelet dysfunction and increased fibrinolysis, there may be a clinical advantage in using lower doses of heparin with heparin-coated surfaces, particularly in relation to the reduction of postoperative blood loss. In their discussion, the authors categorically reject this option. However, the trends in the hematologic parameters they reported, in the face of their relatively small patient sample size, did not justify this rejection. The efforts to evaluate this potentially attractive clinical option need not be stymied by the data provided.

Dr. Gorman, and his colleagues have initiated a healthy scientific discourse that should improve our understanding of the biology of heparin-coated circuitry.

Dr. Gorman. You raise three concerns: the chance the null hypothesis will be accepted when in fact it is false (type II error), the impact of hemodilution, and the impact of hypothermia on our measurements. Although relatively small sample size does reduce the power of statistical calculations for detecting type II errors, moderately low power values do not establish a high probability, for example $p < 0.05$, that two means are different. In other words, a power value of 0.5 means there is a 50% chance that two means are different, but that chance is far too low to conclude that there is a difference. Heparin-coated circuits bear the burden of proving efficacy; otherwise we are discussing theology and not science. When one looks at all of our data and particularly data taken at the end of bypass, when power values are generally high despite small sample sizes, one does not see any evidence that heparin-coated circuitry reduces thrombin formation or activity. This is our primary message; heparin coatings may or may not alter other blood constituents, but our data show no clear effect on thrombin formation and activity.

Correction for hemodilution raises reported values above concentrations that are actually circulating. Corrections for hemodilution are more important for within-group changes than for between group comparisons because the degree of hemodilution is the same in both groups. We have added hematocrit values to the published table to enable interested parties to do their own corrections.

We have no data regarding the efficacy of heparin-coated perfusion circuits during normothermic perfusions.

Dr. George J. Magovern *(Pittsburgh, Pa.).* At an activated clotting time of 400 seconds or longer, it appears that the Carmeda coating confers no benefit with regard to thrombin resistance. Allegheny General Hospital has had some spectacular successes using this coating in the setting of postcardiotomy shock, and we most recently were able to use the coating and the device for 14 days in a venoarterial mode for a patient who had lung graft failure after a double lung transplant, and he recovered and has survived. We have used the coating and the device with a much lower activated clotting time, and I am wondering whether there would have been significant differences between the groups if you had monitored your two groups with lower activated clotting times.

Dr. Gorman. That is a possibility, but we chose not to reduce activated clotting times until we had more evidence that heparin-coated surfaces impair blood clotting.

Dr. Ronald C. Hill *(Morgantown, W.Va.).* These are interesting data. We have had extensive experience with this system clinically and used it with low heparin activated clotting times, activated clotting times in the range of 200 seconds, and have had no clinical problems with it. My questions are as follows: Did you use a pump suction or cardiotomy in this system and did you recirculate after weaning from CPB? If you did use suction or cardiotomy or did not recirculate, you may have stimulated the coagulation complement system. Indeed, we discontinue CPB at about 2 l/min and then immediately recirculate. Also, did you use a biopump? I noticed that you used the system in only four or five coronaries, which is a closed system. Everything else you used was an open system. I would be interested in how you devised your vent system to eliminate the air interface.

Dr. Edmunds. We used both cardiotomy and vent suction produced by roller pumps and a cell salvage system using vacuum suction. We did not recirculate during weaning of CPB or afterwards. All patients received full heparinization, and it is unlikely that recirculation would alter our results in any way.

Dr. Ludwig K. von Segesser *(Zurich, Switzerland).* Unfortunately, CPB today does not consist only of CPB. A number of systems are added to it: a blood cardioplegia system, cardiotomy suction and/or cell salvage devices, hemofilters, and others. My questions to the authors are these: What type of cardioplegia did you use in your study? How did you measure the volume retransfused to the patient? In the coated group four patients had valve type surgery, in which we have to expect much more retransfusion volume, so the comparison could be difficult. Finally, I find that in your results the thrombin-antithrombin complex values, for instance, are quite different for the first 30 minutes of CPB and change afterward. That is just the time at which we normally start to use cardiotomy suction, or, in your case, a cell salvage device.

Dr. Edmunds. We agree completely that the modern heart-lung machine is composed of many subsystems to which blood is exposed. In this study all of these systems were heparin coated in the experimental group except for the Cell Saver System. Only washed red cells were returned from this system. We used antegrade and retrograde cold blood cardioplegia and made no attempt to measure reinfused volumes. We agree that the qualitatively lower thrombin-antithrombin complex values at 5 and 30 minutes in patients in whom coated circuits were used and the demonstration that antithrombin III is adsorbed onto these surfaces suggest that the surface is binding thrombin. The F1.2 values, however, indicate no change in thrombin formation. These data raise the question of whether heparin-coated surfaces are able to release the thrombin-antithrombin complex and whether they become saturated with bound thrombin over time.