

Hemostatic Activation and Inflammatory Response during Cardiopulmonary Bypass

Impact of Heparin Management

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Background: Cardiac surgery involving cardiopulmonary bypass (CPB) leads to fulminant activation of the hemostatic-inflammatory system. The authors hypothesized that heparin concentration-based anticoagulation management compared with activated clotting time-based heparin management during CPB leads to more effective attenuation of hemostatic activation and inflammatory response. In a randomized prospective study, the authors compared the influence of anticoagulation with a heparin concentration-based system (Hepcon HMS; Medtronic, Minneapolis, MN) to that of activated clotting time-based management on the activation of the hemostatic-inflammatory system during CPB.

Methods: Two hundred elective patients (100 in each group) undergoing standard cardiac surgery in normothermia were enrolled. No antifibrinolytic agents or aprotinin and no heparin-coated CPB systems were used. Samples were collected after administration of the heparin bolus before initiation of CPB and after conclusion of CPB before protamine infusion.

Results: There were no differences in the pre-CPB values between both groups. After CPB there were significantly higher concentrations ($P < 0.05$) for heparin and a significant reduction in thrombin generation (25.2 ± 21.0 SD vs. 34.6 ± 25.1), D-dimers (1.94 ± 1.74 SD vs. 2.58 ± 2.1 SD), and neutrophil elastase (715.5 ± 412 SD vs. 856.8 ± 428 SD), and a trend toward lower β -thromboglobulin, C5b-9, and soluble P-selectin in the Hepcon HMS group. There were no differences in the post-CPB values for platelet count, adenosine diphosphate-stimulated platelet aggregation, antithrombin III, soluble fibrin, Factor XIIIa, or postoperative blood loss.

Conclusion: Compared with heparin management with the activated clotting time, heparin concentration-based anticoagulation management during CPB leads to a significant reduction of thrombin generation, fibrinolysis, and neutrophil activation, whereas there is no difference in the effect on platelet activation. The generation of fibrin even in the presence of high heparin concentrations most likely has to be attributed to the reduced antithrombin III concentrations or reduced inhibition of clot-bound thrombin. Therefore, in addition to maintenance of higher heparin concentrations, monitoring and substitution of antithrombin III should be considered to ensure more efficient antithrombin activity during CPB.

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CARDIAC surgery involving cardiopulmonary bypass (CPB) induces powerful activation of the hemostatic and inflammatory systems. This is caused by the contact of blood with the large nonendothelial surfaces of CPB concurrent with the massive activation of the extrinsic pathway of coagulation by the release and reinfusion of tissue factor. In addition to the direct activation *via* the "contact system," thrombin and activated platelets stimulate the inflammatory response.^{1,2} As a result, plasma coagulation factors and platelets are progressively consumed, and the inflammatory response is triggered. Clinical consequences are hemorrhage and the necessity for blood transfusion, which carries a risk of viral and bacterial infections, development of a systemic inflammatory response syndrome, and organ failure.^{3,4}

Intensive efforts have been made to reduce hemostatic activation and inflammation during CPB, including the use of antifibrinolytic agents, the kallikrein inhibitor aprotinin, and coated systems.⁵⁻⁷

However, unfractionated heparins are the key to anticoagulation management during CPB, and few controlled trials have been pursued to determine the optimal heparin management strategy.

Heparin management during CPB commonly employs the activated clotting time (ACT), a marker of inhibition of contact activation. In contrast, the Hepcon heparin management system (HMS; Medtronic, Minneapolis, MN) is based on the measurement and maintenance of individually calculated target heparin concentrations.^{8,9} Although this device has been available for many years, its use in clinical routine is still the exception. There are several studies with regard to this system, but most of them address only partial aspects such as clinical outcome, agreement between heparin anti-Xa activity and Hepcon HMS results, use in pediatric CPB, protamine-heparin ratios, or determination of unfractionated heparin reversal after CPB.¹⁰⁻¹⁵ One study provided a deeper insight into differences in hemostatic activation during CPB comparing the Hepcon HMS and an ACT-based anticoagulation strategy. However, this investigation included only 31 patients (16 treated and 15 control patients) who were scheduled for complex surgery and was thus restricted to a selected patient population and limited in scope.¹⁶

In the current investigation, we compared heparin management using the Hepcon HMS with an ACT regimen by assessing key parameters of coagulation and

inflammation in 200 patients undergoing elective standard cardiac surgery.

Materials and Methods

Groups and Points of Measurement

After obtaining approval by the local ethics committee and informed consent, we enrolled 200 patients undergoing elective standard cardiac surgery (no reoperations, no combined procedures, only operations in normothermic CPB) in this controlled, prospective, randomized investigation. Two groups with 100 patients each were formed. No patient had had coumadine or antiplatelet therapy within 10 days before surgery. In the ACT group, heparin management was performed according to an ACT regimen; in the Hepcon group, the Hepcon HMS was used according to the standard protocol for the device at our institution.

Samples were collected 10 min after administration of the heparin bolus before CPB and after termination of CPB before protamine infusion.

Anesthesia and Cardiopulmonary Bypass

Anesthesia was performed using a total intravenous technique with midazolam, propofol, sufentanyl, and pancuronium bromide on demand. Normothermic CPB was accomplished with "closed" non-heparin-coated systems and the use of membrane oxygenators and roller pumps. Priming of the CPB system was accomplished with 1,500 ml Ringer's solution. In none of the patients were antifibrinolytic agents or the kallikrein inhibitor aprotinin administered. In addition, a cell saver reservoir was used to collect blood from the operation field. This blood was processed (CATS; Fresenius, Bad Homburg, Germany) if the collected volume exceeded 800 ml.

Anticoagulation Protocols

Activated Clotting Time Management. A fixed bolus of heparin (Liquemin; Roche, Grenzach-Wyhlen, Germany) of 300 IU/kg was given, and the kaolin ACT (Hepcon HMS ACT; Medtronic) was performed. The target value for the ACT was 480 s. If this value was not achieved, an additional 10,000 IU heparin was administered until the ACT was prolonged above the target value. In addition, 10,000 units of heparin was added to the CPB circuit. During CPB, an additional 5,000 IU of unfractionated heparin was administered only if the ACT value decreased below the target value. The protamine value was defined 1:1 according to the initial patient heparin bolus (necessary to achieve the target ACT). After infusion of the total CPB volume, an ACT was measured. If the value exceeded 140 s, an additional 50 mg of protamine was given. In cases of decrease of the ACT but prolongation above 140 s, an additional 50 mg protamine was given until the ACT no longer decreased.

Hepcon Management

The Hepcon HMS has been the standard HMS at our institution (> 3,000 CPB/yr) for 4 yr. During this period, we have developed an algorithm for the device to provide rational use of the system and to avoid excessive measurements and costs.

A kaolin ACT of 480 s was determined as target value of the heparin dose-response cartridge for individual calculation of the heparin concentration necessary to achieve this ACT.

The heparin dose-response was performed before skin incision. However, as calculation of the volume of a patient according to body surface area is an approximation, particularly in cardiac surgery, the "pump" heparin was added to the patient bolus to create a safety window. The CPB circuit was primed with an additional 10,000 units of heparin. After administration of the heparin, an ACT (Hepcon HMS kACT) was obtained before initiation of CPB to ascertain the effect of the heparin bolus on inhibition of contact activation. If the ACT was not prolonged above 480 s, an additional 10,000 IU heparin was given. The first determination of the heparin concentration was performed after 30 min using the six-channel heparin-protamine titration cartridge. If additional heparin had to be given to maintain the target concentration, double the required value was given to reach the target value from the "top" and to provide a wider window for further measurements, which were then performed at intervals of 60 min.

After conclusion of CPB, the protamine dose necessary to reverse patient and CPB heparin was calculated according to the results of the heparin-protamine titration measurement. After protamine administration and infusion of the total volume of the CPB circuit, residual free heparin was measured with a low-range heparin-protamine titration cartridge and reversed with protamine.

Transfusion Guidelines

A hemoglobin concentration less than 8.0 mg/dl was determined as the critical level for the transfusion of packed erythrocytes. The trigger for the transfusion of fresh frozen plasma and random donor platelet concentrates was based on the clinical decision of the anesthetist. A postoperative hemorrhage of greater than 1,500 ml within the first 12 h, according to the departmental standards, was evaluated as an indication for surgical reexploration.

Laboratory Analysis

Platelet count was measured using the Cell Dyn 3500R (Abbott, Wiesbaden, Germany). Monitoring of platelet function was performed with 20 μ M adenosine diphosphate-stimulated platelet aggregometry (Mölab; Bio-Data Corporation, Philadelphia, PA) in platelet-rich plasma (heparinized samples). Platelet-rich plasma was prepared by centrifugation at 800 r/min for 15 min, adjusted to a

platelet count of approximately 200,000/ μl , and aggregation was measured at a stir rate of 900 r/min at room temperature. β -thromboglobulin was measured using the Asserchrom β -thromboglobulin enzyme-linked immunosorbent assay (Roche Diagnostics, Mannheim, Germany). Heparin anti-Xa activity was determined using the STA-Rotachrom Heparin (Roche Diagnostics). Antithrombin III was determined using the Coamatic LR Antithrombin Chromogenix (Hemochron Diagnostica, Essen, Germany). Thrombin was measured using the Thrombin/Antithrombin-Complex Enzygnost TAT micro (Dade Behring, Schwalbach, Germany). Soluble fibrin was measured with the COATEST[®] Soluble Fibrin (Chromogenix, Milano, Italy). The Factor XIIIa ELISA (Progen, Heidelberg, Germany) was used to measure Factor XIIIa. D-dimers were measured using the STA-Liatest D-Di (Roche), and neutrophil elastase was measured using a luminescent assay (Auto-ClinLumat LB 952 T; Berthold, Wildbad, Germany). C5b-9 was determined using the SC5b-9 complex ELISA (Innogenics, Heiden, Germany), and soluble P-selectin was measured using the soluble P-selectin ELISA (Biozol, Eching, Germany). The 12-h postoperative blood loss was also determined.

Statistical Analysis

Calculation of sample size, based on a desired decrease in the key parameter of thrombin generation (*i.e.*, thrombin-antithrombin complexes) by 25%, an expected SD (from previous thrombin-antithrombin complexes measurements in our patients) of 75% of the mean value, and a power of 0.9 at $\alpha = 0.05$, resulted in 191 patients. Therefore, 200 patients were included in the study. Statistical analysis of the laboratory data and transfusion requirements was performed using Student *t* test. The reexploration rates were analyzed using the Fisher exact test. A *P* value < 0.05 was determined as significant. The values are expressed as mean \pm SD. The Gaussian normal distribution of the obtained values was assessed using the Kolmogorov-Smirnov test (Statistical Package for Social Science [SPSS] 10.0. for Windows; SPSS Inc., Chicago, IL).

Results

Biometric Data, Surgery, Duration of Cardiopulmonary Bypass, and Heparin Requirements

There were 45 female and 55 male patients in the ACT group and 41 female and 49 male patients in the Hepcon HMS group. The age ranged from 34 to 85 yr, with a mean of 64 ± 13 yr, in the ACT group and 40 to 89 yr, with a mean of 66 ± 17 yr, in the Hepcon HMS group. In the Hepcon group 74% of patients, compared with 69% in the ACT group, had intravenous or subcutaneous heparin therapy before surgery. In the ACT group, cor-

Table 1. Values Obtained before Cardiopulmonary Bypass

	ACT	Hepcon*	<i>P</i> Value
Platelets			
Platelet count ($10^3/\mu\text{l}$)	158.9 \pm 48.7	149.8 \pm 45	0.74
ADP platelet aggregation (%)	82.5 \pm 10.7	81.5 \pm 10.7	0.91
β -Thromboglobulin (IU/ml)	23.5 \pm 5.1	24.1 \pm 4.9	0.79
Plasma coagulation			
Anti-Xa (IU/ml)	3.8 \pm 0.8	4.1 \pm 0.7	0.62
Antithrombin III (%)	86.5 \pm 7.6	89 \pm 6.9	0.68
Factor XIIIa (ng/ml)	1.4 \pm 0.3	1.6 \pm 0.2	0.72
TAT ($\mu\text{g/l}$)	3.5 \pm 0.6	3.7 \pm 0.5	0.81
Soluble fibrin (ng/ml)	10.3 \pm 2.2	10.3 \pm 2.0	0.91
D-dimer ($\mu\text{g/ml}$)	0.2 \pm 0.13	0.3 \pm 0.16	0.74
Inflammation			
Neutrophil elastase (ng/ml)	51 \pm 10.4	49 \pm 11.6	0.76
C5b-9 (ng/ml)	63 \pm 11.2	71 \pm 9.3	0.54
P-selectin (ng/ml)	15.4 \pm 2.4	16.5 \pm 2.0	0.64

* Hepcon heparin management system; Medtronic, Minneapolis, MN.

ACT = activated clotting time; ADP platelet aggregation = adenosine diphosphate-stimulated platelet aggregation; TAT = thrombin-antithrombin complexes.

onary artery bypass grafting (CABG) was performed in 64 patients and valve replacement or reconstruction was performed in 36. In the Hepcon HMS group, 59 patients underwent coronary artery bypass grafting, and 41 underwent valve replacement or reconstruction. The duration of CPB ranged from 66 to 125 min, with a mean of 77 ± 17 min, in the ACT group and from 69 to 145 min, with a mean of 81 ± 21 min, in the Hepcon HMS group. The total amount of heparin given ranged from 300 to 425 IU/kg in the ACT group, with a mean of 354 ± 31 IU/kg, and from 453 to 1,125 IU/kg, with a mean of 657 ± 135 IU/kg, in the Hepcon HMS group (*P* = 0.011).

Laboratory Data

All data were distributed to a Gaussian normal distribution.

Pre-Cardiopulmonary Bypass Data

There were no significant differences in the data between both groups before CPB. Results are given in table 1.

Post-Cardiopulmonary Bypass Data

The values obtained after CPB are given in table 2.

Total 12-h Blood Loss and Transfusions

The 12-h postoperative blood loss was 765 ± 397 ml in the ACT group and 625 ± 312 ml in the Hepcon HMS group (*P* = 0.11). There were 0–4 U of erythrocytes (mean, 0.3 ± 0.15) transfused in the ACT group and 0–3 U (mean, 0.2 ± 0.1) in the Hepcon HMS group (*P* = 0.49). Moreover, 0–3 U of fresh frozen plasma (mean, 0.3 ± 0.1 U) were given in the ACT group and 0–3 U (mean, 0.2 ± 0.13 U) in the Hepcon HMS group (*P* = 0.39). No random donor platelet concentrates were transfused.

Table 2. Values Obtained after Cardiopulmonary Bypass

	ACT	Hepcon*	P Value
Platelets			
Platelet count ($10^3/\mu\text{l}$)	125 \pm 45.8	121.6 \pm 48.7	0.69
ADP platelet aggregation (%)	76.8 \pm 12.8	73.3 \pm 13.3	0.57
β -Thromboglobulin (IU/ml)	267.8 \pm 134	242 \pm 131	0.18
Plasma coagulation			
Anti-Xa (IU/ml)	2.17 \pm 0.9	2.9 \pm 0.9	< 0.01
Antithrombin III (%)	46.2 \pm 10.4	46.1 \pm 11.2	0.96
Factor XIIa (ng/ml)	2.0 \pm 0.9	1.9 \pm 0.8	0.62
TAT ($\mu\text{g/l}$)	34.6 \pm 25.1	25.2 \pm 21.0	0.004
Soluble fibrin (ng/ml)	16.2 \pm 4.6	15.6 \pm 4.2	0.35
D-dimer ($\mu\text{g/ml}$)	2.58 \pm 2.1	1.94 \pm 1.74	0.02
Inflammation			
Neutrophil elastase (ng/ml)	856.8 \pm 428	715.5 \pm 412	0.02
C5b-9 (ng/ml)	786.4 \pm 442	713 \pm 383	0.22
P-selectin (ng/ml)	112.1 \pm 59.6	99.2 \pm 54.3	0.11

* Hepcon heparin management system; Medtronic, Minneapolis, MN.

ACT = activated clotting time; ADP platelet aggregation = adenosine diphosphate-stimulated platelet aggregation; TAT = thrombin-antithrombin complexes.

Outcome

The mean duration of ventilation was 6.5 ± 1.5 h in the ACT group and 5.3 ± 1.2 h in the Hepcon HMS group ($P = 0.24$). All patients were discharged from the intensive care unit on the first postoperative day. Five patients in the ACT group required surgical reexploration because of postoperative hemorrhage. In three of these patients, surgical reasons for this hemorrhage were found. In two of them, hemorrhage was attributed to increased bleeding from the sternum. In the Hepcon HMS group, three patients needed reexploration because of increased hemorrhage. In all patients a surgical reason was found ($P = 0.464$). All patients, except for one from the ACT group who needed re-fixation of the sternum, were discharged from hospital on schedule without complications.

Discussion

After introduction of the Hepcon HMS as the standard anticoagulation strategy in our hospital and after having gained experience with this device, we reported on changes in central clinical outcome data, compared with the previously used ACT-based strategy.¹⁷ These retrospective data revealed a marked reduction of the reexploration rate and postoperative hemorrhage after introduction of the Hepcon HMS. However, the underlying pathobiochemical mechanisms remained unclear.

The current investigation was performed to assess these mechanisms. Our data suggest that a heparin concentration-based anticoagulation management, compared with an ACT-based regimen, does not differ in the effect on platelet activation but is associated with a significant reduction of thrombin generation, fibrinolysis, and neutrophil activation. Fibrin generation, however, was not reduced.

The ACT is performed with celite or kaolin as activators, which are both potent stimulators of the factor XIIa-mediated intrinsic system of coagulation, the "contact system." Our data show that both the ACT- and Hepcon HMS-based heparin management strategies effectively inhibit activation of factor XIIa, as evidenced by the comparable pre- and post-CPB values in both groups (tables 1 and 2).

In contrast, management with the heparin concentration-based system was associated with a significant decrease of D-dimers, indicating enhanced inhibition of fibrinolysis and a significant reduction of thrombin generation (tables 1 and 2). In view of the inhibition of the intrinsic pathway in both groups, the reduced thrombin generation is most likely caused by an enhanced inhibition of the extrinsic "tissue factor-stimulated pathway."

Heparin and antithrombin III reveal an inhibiting effect on activation of the factors XII, XI, X, IX, and II (thrombin) of the plasma coagulation cascade. However, recent investigations have suggested an additional antithrombin III-independent antithrombotic effect of heparins, caused by the endogenous release of tissue factor pathway inhibitor TFPI.¹⁸ This mode of action may provide an explanation for our observation that, despite the significant reduction of thrombin generation in the Hepcon HMS group, the values for soluble fibrin were almost comparable: the maintenance of the higher heparin concentrations using the Hepcon HMS may ensure more effective inhibition of thrombin generation *via* this "TFPI pathway," while the antithrombin III-dependent anti-IIa activity, because of the deficiency of antithrombin III caused by its consumption during perfusion, remains less effective.

Particularly in prolonged CPB, further decrease of the antithrombin III concentrations can be expected. Therefore, the development of a functional point-of-care antithrombin III cartridge is needed. Based on the information of such a test, adequate substitution of antithrombin III could be initiated readily to provide more effective antithrombin activity. However, as an insensitivity to heparins may not only be caused by an antithrombin III deficiency, a rapid monitoring of fibrin formation as an endpoint parameter of "sufficient anticoagulation" is highly desirable. In the view of the upcoming clinical use of direct antithrombin III-independent thrombin inhibitors, such as r-hirudin, the supplementation of these agents to unfractionated heparin in cases of heparin resistance may be an interesting option.

In addition to reducing hemostatic activation, management with Hepcon HMS was associated with a significant reduction of the concentrations of neutrophil elastase and a trend toward lower plasma concentrations of soluble P-selectin, a platelet- and endothelial cell-borne adhesion molecule propagating leukocyte and platelet adhesion to endothelial cells. These data indicate attenuation of neutrophil activation. Platelets and thrombin

play a pivotal role in the activation and modulation of neutrophil action.¹⁹⁻²² Since platelet activation was comparable in both groups, this differential effect on the inflammatory system may be attributed to the reduced thrombin generation in the Hepcon HMS group. The trend toward lower plasma concentrations of C5b-9, a membrane attack complex of the complement system, suggests an additional beneficial effect of the maintenance of higher heparin concentrations on the inflammatory response.

The current investigation aimed to assess differences in the pathobiochemical mechanisms of the two anticoagulation strategies. Therefore, to achieve more standardized and comparable conditions, only elective patients for short, standard CPB procedures were enrolled. In such selected cases, complication rates are lower. This may explain why the outcome data did not reach the significance that has been reported in previous investigations.^{16,17}

We conclude that heparin management according to the heparin concentration-based Hepcon HMS leads to a significant attenuation of hemostatic activation. This effect of the maintenance of higher heparin concentrations during CPB may be related to an antithrombin III-independent mode of action of heparins caused by the endogenous release of TFPI. Since fibrin formation was not completely inhibited despite these high heparin concentrations, supplementation of antithrombin III or a direct thrombin inhibitor may be necessary to achieve sufficient antithrombin activity. Further investigations addressing this subject are necessary. In addition, the current investigation provided new insights regarding the effect of heparin management on the inflammatory response to CPB. The clinical implications of reduced inflammation using the heparin concentration-based anticoagulation strategy need further characterization.

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