THE EFFECTS OF HEPARIN AND EXTRACORPOREAL CIRCULATION ON PLATELET COUNTS AND PLATELET MICROAGGREGATION DURING CARDIOPULMONARY BYPASS

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Background: Cardiopulmonary bypass is associated with platelet activation and reduced platelet counts. Platelet activation may artifactually lower platelet counts by causing aggregation. In vivo platelet activation may increase existent platelet microaggregation ex vivo. We studied platelet counts and existent platelet microaggregation at different stages of cardiopulmonary bypass.

Methods: Twenty-one patients were studied before and after heparinization ($300 \text{ U} \cdot \text{kg}^{-1}$) and at the end of cardiopulmonary bypass. Unaggregated (or single) platelets were counted in hirudin-anticoagulated blood, and total platelets were counted in ethylenediaminetetraacetic acid-anticoagulated blood.

Results: The total platelet count, $198 \pm 61 \times 10^9 \cdot \text{L}^{-1}$, was unaffected by heparin and stayed at $197 \pm 60 \times 10^9 \cdot \text{L}^{-1}$ ($P = .7$) but fell during extracorporeal circulation; the hemodilution-corrected count was $163 \pm 52 \times 10^9 \cdot \text{L}^{-1}$ ($P = .0004$). Heparinization reduced the unaggregated platelet count from (mean ± 1 SD) $178 \pm 62 \times 10^9 \cdot \text{L}^{-1}$ to $155 \pm 60 \times 10^9 \cdot \text{L}^{-1}$ ($P = .0001$). Extracorporeal circulation had little additional effect. The hemodilution-corrected count was $142 \pm 48 \times 10^9 \cdot \text{L}^{-1}$ ($P = .6$).

Conclusions: Heparinization caused platelet activation and increased existent platelet microaggregation ex vivo. During extracorporeal circulation, there was a reduction in total platelets that was greater than could be explained by hemodilution alone, but the unaggregated platelet count did not change significantly when corrected for hemodilution. Furthermore, the increased platelet microaggregation observed after heparinization was no longer evident after this loss. These findings suggest that during extracorporeal circulation, the platelets that formed into microaggregates after heparinization were lost from the circulation in preference to single platelets. (J Thorac Cardiovasc Surg 2000;120:538-43)

The effects of cardiopulmonary bypass on the platelet are still not fully understood, but platelet dysfunction,¹ platelet activation,² and platelet loss³ all occur.

Platelet activation is a tightly regulated series of events that culminates in platelet secretory release and aggregation. Platelets respond to stimulation in 3 phases: shape change, microaggregation (primary aggregation), and macroaggregation (secondary aggregation). Platelet counts performed with anticoagulants that preserve microaggregates (eg, hirudin) are the unaggregated or single platelet count because hematologic counters do not count aggregates as platelets. The total platelet count can be obtained by using an in vitro anticoagulant that causes disaggregation (eg, ethylenediaminetetraacetic acid [EDTA]).

We have demonstrated that in vivo heparinization reduces the platelet count in hirudin-anticoagulated blood^{4,5} by increasing existing platelet microaggrega-

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tion ex vivo.⁴ After correction for hemodilution, no further reduction was observed at the termination of extracorporeal circulation in the earlier of these studies.⁵ The aim of the current study was to investigate the effects of heparin and of extracorporeal circulation on platelet counts and on existent platelet microaggregation during cardiopulmonary bypass.

Methods

This study was approved by the Royal Infirmary of Glasgow Research Ethics Committee (Project No. 97SC001 [896] on May 7, 1997). Informed consent was obtained from all subjects studied. Patients undergoing primary operations with cardiopulmonary bypass were studied. Patients with diabetes receiving warfarin sodium and those dependent on intravenous nitrates or heparin were excluded. Sixteen men and 5 women were recruited (total patients, 21); median age was 63 years (range, 38-79 years). Thirteen patients had isolated coronary revascularization, 5 had isolated single valve replacement, 2 had coronary revascularization and a single valve replacement, and 1 had an ascending aortic aneurysm replaced.

Anesthetic premedication was with temazepam, and induction was with propofol. Opiate analgesia was used, and maintenance anesthesia was with propofol infusion. The anticoagulant used was unfractionated sodium heparin of porcine mucosal origin ($300 \text{ U} \cdot \text{kg}^{-1}$; Leo Laboratories, Risborough, United Kingdom) given through a central venous cannula or directly into the right atrium just before cannulation. Anticoagulation was monitored by activated clotting time with a Hemochron whole-blood coagulation system model 401 (International Technidyne Corporation, Metuchen, NJ). If the activated clotting time was less than 400 seconds, more heparin was given. The cardiopulmonary bypass circuit consisted of an Avecor tubing set, an Affinity 40- μm arterial line filter (Avecor Ltd, Bellshill, Strathclyde, United Kingdom), and a membrane oxygenator (Duo-COBE; COBE Laboratories, Division of Gambro BCT, Stockholm, Sweden) driven by a Stöckert roller pump (Stöckert, Munich, Germany). The pump prime consisted of 2.0 L of Ringer's lactate solution, 50 mmol/L NaHCO_3 , and 8000 U of sodium heparin. Flows were maintained at $2.4 \text{ L} \cdot \text{min}^{-1} \cdot \text{m}^{-2}$. Ringer's lactate solution was used to maintain pump reservoir volume. If the hematocrit level fell below 25%, packed red cells were given. The alpha-stat protocol was used for pH management. The heart was arrested with either antegrade crystalloid (St Thomas' Hospital No. 1 solution) or blood cardioplegia. Core temperature ranged between 28°C and 33°C . Cardiopulmonary bypass lasted from 57 to 140 minutes (mean, 84 minutes).

Blood samples were taken from the radial artery line just before and 5 minutes after heparin administration but before the onset of extracorporeal circulation. A third sample was taken at the end of cardiopulmonary bypass before heparin reversal. This covered the period of greatest platelet loss,^{2,3} while remaining free of the effects of protamine sulfate on

Table I. Hemoglobin concentrations

Anticoagulant	EDTA	Hirudin
Before heparin ($\text{g} \cdot \text{dL}^{-1}$)	11.5 ± 1.5	11.4 ± 1.4
After heparin ($\text{g} \cdot \text{dL}^{-1}$)	11.3 ± 1.6	11.2 ± 1.8
End cardiopulmonary bypass ($\text{g} \cdot \text{dL}^{-1}$)	$7.9 \pm 1.1^*$	$8.0 \pm 1.1^*$

Data are shown as means $\pm$ 1 SD.

* $P < .0001$ for end cardiopulmonary bypass versus after heparin administration.

platelet counts.⁶ At each sampling, after the dead space in the sampling line had been cleared, the next 2 mL of blood was discarded. The samples were divided: one part was anticoagulated with hirudin (final concentration, $200 \text{ U} \cdot \text{mL}^{-1}$) and the other with EDTA (5 mmol/L).

Materials. Recombinant desulphatohirudin HV1 (r-hirudin, CGP 39393) with a specific activity of $11.7 \text{ ATE} \cdot \text{mg}^{-1}$ (antithrombin units per milligram) was dissolved in 0.9% saline solution containing 0.1% polyethylene glycol 600 at a concentration of $20,000 \text{ U} \cdot \text{mL}^{-1}$. Fifteen microliters of this solution was stored in 1.5 mL of Eppendorf microtubes at -20°C and thawed just before use. Siliconized glass tubes were used for the EDTA-anticoagulated samples.

Platelet counting and platelet aggregate ratios. Platelets were counted in whole blood by use of a Coulter T660 counter (Coulter Corporation, Hialeah, Fla). This instrument also determined the hemoglobin content, which was used to correct for hemodilution. The hemoglobin was used in preference to the hematocrit level because the Coulter T660 counter measures the hemoglobin and uses this value to calculate the hematocrit.

There was no difference between the hemoglobin concentrations of EDTA- and hirudin-anticoagulated blood at any of the 3 sampling times (Table I). The hemoglobin concentration was unaffected by heparinization ($P = .4$, EDTA; $P = .5$, hirudin; $n = 21$) but fell significantly during extracorporeal circulation (both $P < .0001$; $n = 21$; Friedman analysis of variance). Corrections for hemodilution were made to the individual platelet counts both after heparinization and at the end of cardiopulmonary bypass.

Platelet aggregate ratios were calculated for each individual set of paired platelet counts, as described by Wu and Hoak.⁷ The numerator was the unaggregated or single platelet count obtained from hirudin-anticoagulated blood, and the denominator was the total platelet count obtained from EDTA-anticoagulated blood. This technique measures changes in the in vivo platelet activation state by serial comparisons of existent platelet aggregation ex vivo and is described in detail elsewhere.⁴

Statistical analysis. Data are expressed as means $\pm$ 1 SD. All P values quoted in the results are obtained by the Friedman 2-way analysis of variance. Serial and paired data were also compared by the Wilcoxon signed-rank test to confirm these analyses. All analyses were performed with the use of Arcus Quickstat Biomedical software retailed by Addison Wesley Longman trading as Research Solutions.

Fig 1. Total versus unaggregated hemodilution-corrected platelet counts. *EDTA*, Total platelet count; *hirudin*, unaggregated platelet count; *CPB*, cardiopulmonary bypass.

Results

Uncorrected platelet counts. Platelet counts in hirudin-anticoagulated blood were consistently lower than corresponding counts in EDTA-anticoagulated blood (all $P < .0001$; $n = 21$), implying existent aggregation (Table II).

Total platelets. The total platelet count obtained from EDTA-anticoagulated blood was unaffected by heparinization ($P = .7$; $n = 21$) but fell significantly during extracorporeal circulation ($P < .0001$; $n = 21$; Table II).

Unaggregated platelets. Heparinization per se significantly reduced the unaggregated platelet count in hirudin-anticoagulated blood ($P < .0001$; $n = 21$). During extracorporeal circulation, a further significant fall was seen ($P < .0001$; $n = 21$; Table II).

Dilution-corrected platelet counts

Total platelets. After correction for hemodilution, the total (EDTA-anticoagulated) platelet count, $198 \pm 61 \times 10^9 \cdot L^{-1}$, was unaffected by heparin and stayed at $197 \pm 60 \times 10^9 \cdot L^{-1}$ ($P = .7$ vs before heparin administration; $n = 21$). The count fell during extracorporeal circulation to $163 \pm 52 \times 10^9 \cdot L^{-1}$ ($P = .0004$ vs after heparin administration; $n = 21$).

Unaggregated platelets. Heparinization reduced the dilution-corrected unaggregated (single) platelet count in hirudin-anticoagulated blood from $178 \pm 62 \times 10^9 \cdot L^{-1}$ to $155 \pm 60 \times 10^9 \cdot L^{-1}$ ($P = .0001$ vs before heparin administration; $n = 21$). Extracorporeal circu-

Table II. Summary statistics for uncorrected platelet counts

Sampling time	EDTA	Hirudin
Before heparin ($\times 10^9 \cdot L^{-1}$)	198 ± 61	178 ± 62
After heparin ($\times 10^9 \cdot L^{-1}$)	197 ± 58	$155 \pm 60^*$
End cardiopulmonary bypass ($\times 10^9 \cdot L^{-1}$)	$117 \pm 48^\dagger$	$102 \pm 44^\dagger$

Data are shown as means ± 1 SD.

* $P < .01$ for after heparin administration versus before heparin administration.

† $P < .001$ for end cardiopulmonary bypass versus after heparin administration.

lation had little additional effect. The final count was $142 \pm 48 \times 10^9 \cdot L^{-1}$ ($P = .6$ vs after heparin administration; $n = 21$).

The paired dilution-corrected platelet counts at the 3 time points studied are shown in Fig 1, which demonstrates the individual variations in the extent of platelet aggregation and platelet loss. It also underscores the significance of the differences.

Platelet aggregate ratios. Platelet aggregate ratios were calculated by using the uncorrected counts to avoid errors that may be introduced by approximations used during the corrections. Heparin reduced the platelet aggregate ratio from 0.90 ± 0.08 to 0.78 ± 0.14 ($P < .0001$; $n = 21$). During extracorporeal circulation, the platelet aggregate ratio returned to near baseline at 0.87 ± 0.11 ($P = .0009$ vs after heparin administration and $P = .08$ vs before heparin administration; $n = 21$; Fig 2).

Discussion

The main findings of this study are as follows. First, we confirmed our previous observation that heparinization for cardiopulmonary bypass increases existent platelet microaggregation *ex vivo*.⁴ Second, during extracorporeal circulation, the reduction in total platelet concentration was greater than could be explained by dilution alone, as seen previously.^{2,3} Third, despite the reduction in total platelets, the dilution-corrected unaggregated platelet count did not change significantly. Finally, the loss of existent platelet microaggregates accounted for most of the fall in the dilution-corrected total platelet count during extracorporeal circulation.

Heparin and platelets. The proaggregatory action of heparin may be underrated because intravenous heparin also selectively inhibits platelet macroaggregation,^{5,8,9} limiting the total response of platelets to stimulation. Therefore, although intravenous heparin impairs platelet hemostatic performance, it may inappropriately elevate other platelet functions, such as their inflammatory activity.

Extracorporeal circulation and platelets. The current findings suggest that the more active platelets (ie, those formed into microaggregates) were preferentially lost from the circulating platelet pool during cardiopulmonary bypass. Previous workers have also reported a preferential loss of activated platelets during extracorporeal circulation.¹⁰ Others observed that increased platelet responsiveness to in vitro agonists induced by heparinization was absent after the platelet count fell during extracorporeal circulation.¹¹

Activated platelets may be preferentially sequestered in body organs or deposited on the surfaces of the extracorporeal circuit because they more readily adhere to foreign surfaces or endotheliocytes than resting platelets. Conceivably, platelet adherence would be greatest in areas with low flow rates and a large surface area/volume ratio, such as the hepatic portal circulation. This would explain why the liver^{3,12} and the oxygenator^{12,13} are major sites of platelet accumulation during extracorporeal circulation. Reducing platelet deposition on the extracorporeal circuit by modifying its surface may increase platelet accumulation in the liver and other organs.¹⁴

Activated platelets may contribute to the cytokine production observed in the liver during cardiopulmonary bypass¹⁵ by causing the endotheliocytes to secrete chemokines and adhesion molecules.¹⁶ Platelets sequestered in the body during extracorporeal circulation later return to the circulation and have a normal life span.³ This emphasizes the reversibility of early platelet responses to stimuli and implies that cycles of activation and return to the resting state do not affect platelet longevity.

Hemodilution, which occurs at the onset of extracorporeal circulation, reduces the platelet concentration at the center of the tube more markedly than it does near the wall.¹⁷ Hemodilution also causes marginalization of erythrocytes,¹⁷ which displace the fluid medium and nearby particles, including platelets, thus increasing the interactions of these other particles with each other and the tube walls.¹⁸ Hemodilution may therefore accelerate platelet adherence to vessel and circuit walls, thus explaining the marked decrease in total circulating platelets occurring at the onset of extracorporeal circulation.^{2,3}

These considerations suggest that platelet inhibition would reduce platelet loss if started before heparin administration. In clinical studies at this institution, prostacyclin failed to reduce perioperative bleeding despite reduced platelet activation¹⁹ and deposition on filters.²⁰ Other investigators report similar findings.²¹⁻²³ We suggest that this is because inhibiting platelets pre-

Fig 2. Mean platelet aggregate ratios. Error bars represent 1 SD. CPB, Cardiopulmonary bypass.

serves their numbers, although they remain dysfunctional.

Theoretically, the inhibitory effect of heparin on platelet macroaggregation,^{5,8,9} by preventing irreversible aggregation, may preserve platelets; however, platelet loss during extracorporeal circulation may be largely reversible.³ Furthermore, the current study and others¹⁰ suggest that the loss of circulating platelets during cardiopulmonary bypass may be more closely related to shape change and microaggregation, which precede the macroaggregatory response, which is inhibited by heparin.

Limitations of the study. The effects of heparin and extracorporeal circulation were not studied separately. We were unable to study subjects receiving large doses of heparin and not undergoing extracorporeal circulation. The information gained from in vitro studies of the effects of heparin on blood is limited because some effects of intravenous heparin on platelets are not reproduced by in vitro heparinization.^{5,9,24} Intravenous heparin releases several proteins from the endothelium into plasma.²⁵ Because these proteins may affect platelets, information from studies of simulated extracorporeal circulation by using blood heparinized in vitro should be interpreted cautiously.

The effects of heparin may be separated from those of extracorporeal circulation per se by studies using models of cardiopulmonary bypass comparing heparin with alternative anticoagulants. The near-universal use of heparin to provide anticoagulation for cardiopulmonary bypass hinders this. Hirudin anticoagulation is currently being used for clinical cardiopulmonary bypass in patients with heparin-induced thrombocytopenia type II,²⁶ but these patients are an inappropriate study model because their platelets are already defective. Workers investigating anticoagulation with selec-

tive thrombin inhibitors for cardiopulmonary bypass in animals report better preservation of platelet numbers^{27,28} and reduced platelet deposition on oxygenator membranes²⁷ than with heparin. This may be because selective thrombin inhibitors, like hirudin and argatroban, which have no direct inhibitory actions on platelets, improve platelet preservation by eliminating the stimulatory actions of heparin.

Conclusion

Heparinization caused platelet activation, which increased existent platelet microaggregation *ex vivo*. Dilution did not explain the full decrease in total platelet concentration observed during extracorporeal circulation; the loss of existent platelet microaggregates accounted for most of the deficit. Heparin, by increasing existent platelet microaggregates, may therefore promote platelet loss from the circulating pool during extracorporeal circulation.

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