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Effect of Combined Anticoagulation Using Heparin and Bivalirudin on the Hemostatic and Inflammatory Responses to Cardiopulmonary Bypass in the Rat

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Background: **Despite high-dose heparin anticoagulation, cardiopulmonary bypass (CPB) is still associated with marked hemostatic activation. The purpose of this study was to determine whether a reduced dose of bivalirudin, added as an adjunct to heparin, would reduce thrombin generation and circulating markers of inflammatory system activation during CPB as effectively as full-dose bivalirudin, without adversely affecting postoperative hemostasis.**

Methods: **Using a model of normothermic CPB in rats, the authors prospectively compared markers of thrombin generation (thrombin–antithrombin complexes) and inflammatory markers (tumor necrosis factor α, interleukin 1β, interleukin 6, and interleukin 10) in three groups: conventional high-dose heparin (H), full-dose bivalirudin (B), and a combined group (standard high-dose heparin with the addition of reduced dose bivalirudin or H&B), at baseline, after 60 min of CPB, and 60 min after CPB. Postoperative hemostasis was also assessed.**

Results: **Groups H&B and B showed reduced thrombin–antithrombin complex formation during CPB compared with group H ($P = 0.0003$), and this persisted after CPB for group B ($P = 0.009$). Perioperative increases in interleukin 6 and interleukin 10 showed a trend toward being reduced in animals receiving bivalirudin ($P = 0.06$). Evidence of residual anticoagulation was found in group H&B as measured by activated clotting time ($P = 0.04$) and activated partial thromboplastin time ($P = 0.02$), but no intergroup difference in primary hemostasis was found.**

Conclusions: **Bivalirudin attenuates hemostatic activation during experimental CPB with potential effects on markers of the inflammatory response. However, with this dosing regimen, the combination of heparin and bivalirudin does not seem to confer any measurable advantages over full-dose bivalirudin anticoagulation.**

HEPARIN has long been established as the anticoagulant of choice for cardiac surgery. However, despite high-dose heparin anticoagulation, considerable thrombin-mediated, hemostatic activation still occurs during cardiopulmonary bypass (CPB).[1,2] Hemostatic activation leads to consumption of natural circulating anticoagulants, which may be clinically important, because it has been independently associated with thrombotic complications after cardiac surgery.[3] The coagulation system also exhibits extensive crosstalk with cellular and humoral aspects of the inflammatory response.[4] Inflammatory cytokines increase during cardiac surgery and are associated with an increased incidence of postoperative complications,[5–7] further identifying hemostatic activation as a potential therapeutic target to improve outcome after cardiac surgery.

Ideally, anticoagulation regimens should suppress thrombin generation, because thrombin activates the hemostatic system by cleaving fibrinogen to form fibrin and activating factors V, VIII, XI, and XIII. In addition, by binding to cell surface protease-activated receptors,[8] thrombin activates endothelial cells leukocytes and platelets, inducing the synthesis and release of proinflammatory cytokines *via* mitogen-activated protein kinase and nuclear factor κB–mediated mechanisms.[9] While heparin anticoagulation reduces thrombin generation in a dose-dependent manner[1] and partially inhibits the tissue factor pathway by increasing the synthesis and secretion of tissue factor pathway inhibitor, effective thrombin suppression may require additional anticoagulants.

The direct thrombin inhibitor hirudin is a naturally occurring anticoagulant derived from the medicinal leech and has demonstrated efficacy in models of thrombosis.[10] Hirudin also exhibits antiinflammatory actions by reducing cytokine expression and leukocyte infiltration[11] as well as by suppressing the expression of tissue factor on circulating monocytes.[12] Hirudin's long half-life has led to hemorrhagic complications after use as a heparin alternative for cardiac surgery,[13] but the synthetic 20–amino acid polypeptide bivalirudin (Angiomax®; The Medicines Company, Parsippany, NJ), with a relatively short half life of 30 min, has been safely used during cardiac surgery.[14–16] Bivalirudin comprises an amino-terminal moiety D-Phe-Pro-Arg-Pro- that avidly binds to the active catalytic site of thrombin, a tetraglycine spacer and the carboxy-terminal residues 54–64 of hirudin that antagonize recognition/binding of fibrinogen at the anion binding exosite 1 of thrombin.[17] Bivalirudin possesses a predictable, dose-dependent anticoagulant activity[18] and may therefore be useful either as an alternative or an adjunct to heparin anticoagulation.

The purpose of this study was to use bivalirudin to inhibit thrombin generation during experimental CPB.

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By combining a reduced bivalirudin dose with heparin, we aimed to achieve effective thrombin inhibition, similar to full-dose bivalirudin, while minimizing residual circulating bivalirudin levels. We tested the hypothesis that the addition of bivalirudin to standard-dose heparin anticoagulation would reduce perioperative hemostatic and inflammatory markers without adversely affecting postoperative hemostasis in a rat model of CPB.

Materials and Methods

All animals were treated in compliance with the *Principles of Laboratory Animal Care* formulated for the National Society for Medical Research[19] and the *Guide for the Care and the Use of Laboratory Animals* prepared by the National Academy of Science (National Institutes of Health Publication No. 86-23, revised in 1996).[20] Experimental protocols were approved by the Duke University Animal Institutional Animal Care and Use Committee.

Group Assignments

Sham-operated controls (n = 6) were performed initially, as a pilot study, to determine the baseline thrombin generation and inflammatory response seen with preparation for CPB (n = 6). This group received similar general anesthesia and cannula placement, as described in detail below with anesthesia maintained for a 120-min period to duplicate the 60-min CPB time and 60-min recovery time for the treatment animals.

Subsequently, rats were randomly assigned to undergo CPB with one of three anticoagulation regimens: 350 U/kg porcine heparin (group H, n = 13); 0.8-mg/kg bolus and 2.5 · bivalirudin (group B, n = 13); or 350 U/kg heparin and 0.4-mg/kg bolus and 1 $mg \cdot kg^{-1} \cdot h^{-1}$ bivalirudin (group H&B, n = 13). After the placement of the arterial catheter and before full anticoagulation, rats were given 150 U/kg heparin (group H and H&B) and 50 U/kg heparin (group B) to keep the CPB catheters thrombus free during surgical preparation, as previously described.[21] The two doses of bivalirudin were previously used in a rat thrombosis model[22] and were chosen because they resulted in similar plasma levels to those used clinically.[14,23,24] The higher bivalirudin dose (group B) is similar to that used during CPB,[24] and the lower dose (group H&B) is similar to that used during off-pump, coronary artery bypass grafting surgery.[14,16]

Surgical Preparation and Cardiopulmonary Bypass

Male Sprague-Dawley rats (aged 12–14 weeks; weight, 350–400 g; Harlan, Indianapolis, IN) were fasted for 4 h but had free access to water. Rats were cannulated for CPB as previously described.[21] Briefly, surgery was performed, using aseptic techniques, in anesthetized rats (2–2.5 vol% isoflurane) that were endotracheally intubated (14-gauge intravenous catheter) and mechanically ventilated (45% oxygen–balance nitrogen; partial pressure of carbon dioxide 35–45 mmHg). All surgical fields were subsequently infiltrated with 1% lidocaine. The tail artery was cannulated with a 20-gauge catheter for aortic inflow, and *via* the right external jugular vein, a 4.5-French multiorifice cannula was advanced into the right atrium for venous return. Mean arterial blood pressure was monitored *via* a catheter in the right superficial caudal epigastric artery. Baseline physiologic measurements, including mean arterial blood pressure and rectal temperature, as well as arterial blood gases, were recorded 10 min before commencement of CPB.

The CPB circuit consisted of a venous reservoir, a peristaltic pump (Masterflex®; Cole-Parmer Instrument Co., Vernon Hills, IL), a custom-designed membrane oxygenator, an in-line flow probe (2N806 probe and T208 flowmeter; Transonics Systems, Inc., Ithaca, NY) and an arterial inflow cannula, all of which were connected *via* 1.6-mm-ID silicone tubing (Tygon®; Cole-Parmer Instrument Co.). The custom-designed membrane oxygenator consisted of two Plexiglas® shells (12.8 × 12.8 × 2.7 cm) (Altuglas International, Philadelphia, PA) carrying the hollow fiber diffusion membrane providing a gas exchange area of 558 $cm.^2$ The CPB circuit was primed with 6% hetastarch (3–5 ml) and, in the heparin groups (H and H&B), 50 U heparin was added to the prime.

Rectal temperature was monitored and servo-regulated at 37.5° ± 0.1°C (YSI 400 series thermistor and 73ATA indicating controller; YSI, Yellow Springs, OH) using a heating blanket and convective forced-air heating system. Arterial line inflow temperature was maintained at 37.5°C using a circulating water bath system.

In the treatment groups, animals were subjected to 60 min of normothermic nonpulsatile CPB with flow rates of 160–180 $ml \cdot min^{-1} \cdot kg^{-1}$, which is similar to the normal cardiac output in the rat. For the entire CPB period, ventilation of the lungs was discontinued. After 60 min of CPB, the animals were weaned without the need for inotropic support. After 60 min of recovery from CPB, the animals were killed by exsanguination during deep anesthesia. The sham-operated group had all catheters left in place for the same 120-min period as the treatment groups before euthanasia and received no additional intravenous fluid. Inspired oxygen was maintained between 80% and 100% for all animals.

Blood Sampling

Arterial blood gas analysis was performed using an IL 1306 blood gas analyzer (Instrument Laboratories, Inc., Lexington, MA), and hemoglobin was determined using an OSM3 Hemoximeter® (Radiometer Inc., Copenhagen, Denmark); all analyzers were calibrated to rat blood. Samples of 1.0 ml whole blood were drawn at baseline after cannulae placement, after 60 min of CPB, and after 60 min of recovery and were then immediately centri-

fuged at 3,000 rpm for 15 min in a refrigerated centrifuge. The platelet poor plasma retrieved was stored at −70°C with subsequent enzyme-linked immunosorbent assay methods used for analysis of thrombin–antithrombin (TAT) complexes, tumor necrosis factor α, interleukin 1β, interleukin 6, and interleukin 10 levels (Search Light Inc., Woburn, MA). These markers were chosen because of previous observations of a response in this model,[25] because the generation of thrombin is highly correlated to TAT complex levels, and because the assay performs well with rat blood.[26,27] Samples were drawn into Eppendorf tubes relying on circulating anticoagulants during sample handling.

An additional 15 animals (5 randomized to each group) were subsequently studied to determine the effects of the different anticoagulation regimens on postoperative hemostasis. The doses of anticoagulants given were identical to those described previously, but 1.25 mg protamine sulfate (Eli Lilly, Indianapolis, IN) per 100 U heparin given was infused over half an hour (to avoid hemodynamic instability) after separation from CPB in the H and H&B groups. An equal volume of saline was infused in group B.

Activated clotting times (ACTs) (Actalyke® with Max-Act® tubes; Helena Laboratories, Beaumont, TX) required only 0.5 ml per sample and were measured at baseline and 1 h after separation from CPB after reversal of heparin with protamine. ACTs were not measured during CPB because bivalirudin is not dosed according to the ACT[15] and it was important to minimize blood sampling to avoid excessive hemodilution or hypovolemia. At the final time point only, the tail bleeding time was measured as previously described.[28] Additional plasma from the final time point was prepared from blood drawn directly into syringes containing sodium citrate anticoagulant and frozen at −70°C for batch analysis.

With these samples, we further determined the effects of residual anticoagulation using the following assays: anti–factor Xa levels using a chromogenic assay (Biomerieux, Durham, NC); activated partial thromboplastin time (APTT) and ecarin clotting time (ECT) using standard APTT reagents and 4 U/ml ecarin in 0.2 M HEPES-buffered saline (Sigma, St. Louis, MO) after the addition of 0.02 5M $CaCl_2$ to citrated plasma samples. The clotting endpoint was determined using a fibrometer (BBL Fibrometer; Becton Dickinson and Co., Franklin Lakes, NJ); we determined the range of values in normal, nonanticoagulated rats to be 19–30 s for APTT and 28–45 s for ECT with this system. All samples were measured in duplicate, and the average was used in the statistical analysis.

Statistical Analysis

Physiologic and hematologic parameters were compared across treatment groups using 2-degree-of-freedom Wilcoxon rank sum tests. TAT complex and cytokine values were characterized as the absolute change from baseline (just after cannula insertion) to the end of CPB to account for variation in hemostatic and inflammatory markers occurring during the surgical preparation process. Groups were then compared with the Wilcoxon rank sum test. Measures of postoperative hemostasis were compared with either Wilcoxon tests, when actual values were reported, or chi-square tests comparing incidence of abnormal values, when truncated values such as APTT $>$ 150 s were reported. When a significant difference was observed between treatment groups, *post hoc* pairwise comparisons were performed to identify the source of difference. A P value of less than 0.05 was considered statistically significant. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

Results

All 54 animals survived the procedure and had blood samples drawn at the specified time points; one animal in group H missed anti–factor Xa level measurement because of an insufficient amount of plasma. In group B, intraoperative data for seven animals were unavailable or excluded because of the presence of visible clot in whole blood or fibrin stranding in thawed plasma samples; this problem was subsequently eliminated for the assessment of postoperative hemostasis by drawing samples directly into a syringe containing citrate anticoagulant. The hematologic and physiologic data are presented by treatment group in tables 1 and 2, respectively. In all groups, hemodilution is apparent by the reduced hematocrits and platelets counts during CPB, and a mild acidosis developed during CPB that resolved after CPB. Minor intergroup differences included a higher temperature in group B at the end of CPB and a higher pH in group H&B after CPB (table 2).

With regard to markers of thrombin generation, there was no increase in TAT complex levels in the sham-operated animals during the time simulating the duration of CPB (P = 0.31). The increase in TAT complexes during CPB was greater in group H compared with either group H&B (P = 0.0003) or group B (P = 0.007), as illustrated in figure 1. Postoperatively, after heparin reversal with protamine, TAT complex levels were lower in group B than in either of the heparin groups, as shown in table 3 (P = 0.009).

Comparisons of inflammatory markers between groups identified trends toward lower interleukin-10 (P = 0.056) and interleukin-6 (P = 0.057) levels in animal receiving bivalirudin (figs. 2 and 3), but tumor necrosis factor α (P = 0.12) and interleukin 1β (P = 0.13) showed no treatment effect.

Measures of postoperative hemostasis in 15 subsequently studied, experimental animals are reported in table 3. After heparin reversal with protamine, the ACT remained higher than baseline in group H (P = 0.003) and group H&B (P =

Table 1. Hematologic Parameters by Experimental Group

	Group B	Group H	Group H&B	*P* Value
Baseline				
Hct	40 (37.2–42.8)	39.7 (37.8–43.2)	39.5 (38.4–41.6)	0.99
Plt	836 (788–920)	816 (680–891)	808 (664–876)	0.62
On CPB				
Hct	22.3 (21.4–24.4)	23 (21.4–24.1)	22.7 (19.9–23.8)	0.83
Plt	361 (213–436)	395 (356–492)	404 (342–460)	0.57
After CPB				
Hct	23.6 (22.6–26.4)	22 (20.6–26.2)	21.7 (19.8–22.5)	0.18
Plt	508 (387–608)	440 (400–536)	508 (432–528)	0.73

Values are expressed as median (interquartile range), and *P* values are derived from 2-degree-of-freedom Wilcoxon rank sum tests.

CPB = cardiopulmonary bypass; group B = anticoagulation with high-dose bivalirudin; group H = anticoagulation with standard-dose heparin; group H&B = anticoagulation with standard-dose heparin and low-dose bivalirudin; Hct = hematocrit; Plt = platelet count $\times 10^9$/l.

0.01) but not in group B ($P = 0.82$). Measurement of anti–factor Xa levels provided evidence of equal amounts of residual circulating heparin in groups H&B and H ($P = 0.77$) that were higher than in group B ($P = 0.03$ and 0.014, respectively). However, despite these equal anti–factor Xa levels in groups H and H&B, the ACT, APTT, and ECT values at this time were only significantly prolonged in group H&B compared with group B ($P = 0.03$, 0.01, and 0.049, respectively). However, with regard to primary hemostasis, there was no difference in the *in vivo* tail bleeding time between groups ($P = 0.43$).

Discussion

We demonstrated biochemical evidence of thrombin generation and elevated inflammatory markers during experimental CPB with conventional high-dose heparin anticoagulation; the use of the direct thrombin inhibitor bivalirudin as an adjunct or an alternative to heparin reduced these responses. We accept our hypothesis that combining a low dose of bivalirudin with standard-dose heparin reduces detectable thrombin generation during experimental CPB and note trends toward a reduction in inflammatory markers with bivalirudin anticoagulation.

These observations of reduced thrombin generation with bivalirudin use are in keeping with the additive anticoagulant effect of heparin and bivalirudin. In addition, the ability of full-dose bivalirudin to inhibit clot or cellular bound thrombin is consistent with observations during clinical cardiac surgery.[29] Further, thrombosis models have revealed that markers of thrombin forma-

Table 2. Physiologic Variables by Experimental Group

	Group B	Group H	Group H&B	*P* Value
Baseline				
MAP	66 (63–79)	76 (62–85)	67 (58–77)	0.42
pH	7.44 (7.42–7.49)	7.48 (7.44–7.50)	7.48 (7.45–7.51)	0.51
P_{CO_2}	42 (39–46)	42 (35–45)	39 (34–42)	0.56
BE	6.4 (5.2–7.2)	6.5 (4.2–7.2)	5.8 (4.7–7.5)	0.99
P_{O_2}	125 (111–139)	127 (108–159)	157 (123–174)	0.16
On CPB				
pH	7.18 (7.10–7.28)	7.24 (7.21–7.27)	7.27 (7.19–7.30)	0.46
P_{CO_2}	68 (56–82)	53 (49–68)	56 (48–68)	0.26
BE	−2.2 (−3.8 to −1.1)	−1.3 (−4.4 to −0.1)	−2.8 (−4.7 to 1.7)	0.97
P_{O_2}	376* (301–471)	490 (389–512)	494* (456–529)	0.04
MAP start of CPB	53 (50–62)	60 (55–64)	54 (52–62)	0.15
MAP end of CPB	53 (49–58)	53 (50–58)	51 (50–55)	0.81
Q	64 (61–79)	62 (55–67)	60 (56–67)	0.15
T	37.4*† (37.1–37.6)	36.9† (36.8–37.1)	36.7* (36.3–37.1)	0.005
After CPB				
pH	7.47* (7.42–7.49)	7.41 (7.39–7.44)	7.50* (7.41–7.52)	0.05
P_{CO_2}	41 (38–44)	40 (35–43)	35 (32–41)	0.29
BE	4.8 (4.0–6.1)	1.1 (−2.4 to 4.6)	2.4 (1.8–5.7)	0.07
P_{O_2}	178 (117–280)	246 (86–364)	275 (127–481)	0.72

Values are expressed as median (interquartile range), and *P* values are derived from 2-degree-of-freedom Wilcoxon rank sum tests, with † and * denoting groups that differ after *post hoc*, pairwise Wilcoxon tests ($P < 0.05$).

BE = base excess; CPB = cardiopulmonary bypass; group B = anticoagulation with high-dose bivalirudin; group H = anticoagulation with standard-dose heparin; group H&B = anticoagulation with standard-dose heparin and low-dose bivalirudin; MAP = mean arterial pressure (in mmHg); P_{CO_2} = partial pressure of carbon dioxide (in mmHg); P_{O_2} = partial pressure of oxygen (in mmHg); Q = cardiopulmonary bypass pump flow rate (in milliliters per minute); T = core temperature (in degrees centigrade).

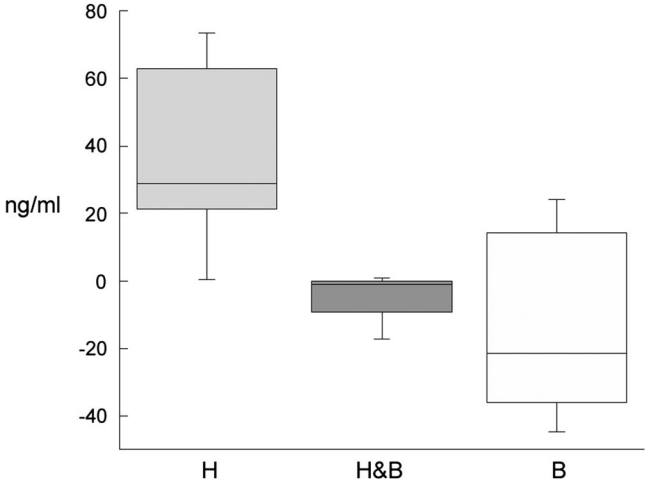

Fig. 1. Thrombin–antithrombin complex formation during cardiopulmonary bypass by treatment group, expressed as the increase from a pre–cardiopulmonary bypass baseline to after 60 min of cardiopulmonary bypass. The *box plot* depicts the median and interquartile range; the *whiskers* represent the 10th and 90th percentiles. There is a marked intergroup difference in thrombin–antithrombin complex formation during cardiopulmonary bypass by Wilcoxon rank sum test ($P = 0.0003$). B = anticoagulation with high-dose bivalirudin; H = anticoagulation with standard-dose heparin; H&B = anticoagulation with standard-dose heparin plus low-dose bivalirudin.

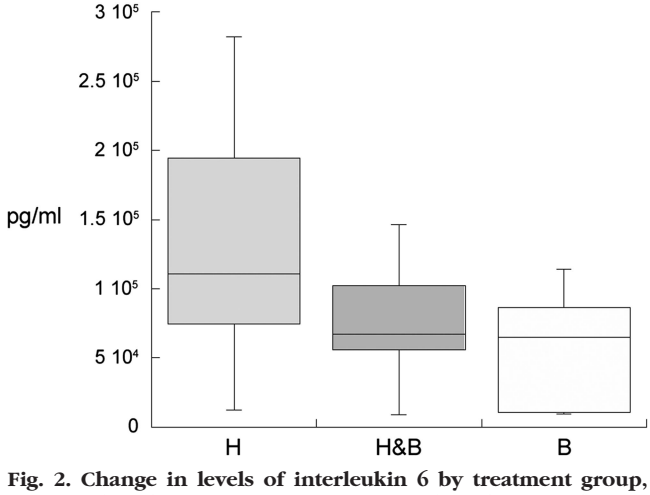

Fig. 2. Change in levels of interleukin 6 by treatment group, measured as the increase from a pre–cardiopulmonary bypass baseline to a time point after a 60-min period of recovery after 60 min of cardiopulmonary bypass. The *box plot* depicts the median and interquartile range; the *whiskers* represent the 10th and 90th percentiles. There is a trend toward an intergroup difference by Wilcoxon rank sum test ($P = 0.058$). B = anticoagulation with high-dose bivalirudin; H = anticoagulation with standard-dose heparin; H&B = anticoagulation with standard-dose heparin and low-dose bivalirudin.

tion and fibrinogenolysis induced by infused thromboplastin can be abolished by hirudin therapy,[10] and lower levels of the fibrinogenolysis marker (fibrinopeptide A) are found in bivalirudin compared with heparin-treated patients with acute coronary syndrome.[23] We postulate that the persistent effect of full-dose bivalirudin on reducing thrombin generation into the post-CPB period may be related to reported trends in reduced myocardial injury and graft failure seen with bivalirudin use.[14,16] This may be a result of avoiding protamine, because protamine administration has been linked to reduced epicardial blood flow.[30]

An effect of bivalirudin on inflammatory markers is feasible, because direct thrombin inhibition may reduce protease activated receptor-1 activation, in turn reducing interleukin-6 release from monocytes,[31] mast cells,[32] and endothelial cells.[33] Avoidance of cardiotomy suction, a potent source of tissue factor and thrombin,[34] reduced interleukin-6 levels measured after cardiac surgery,[35] supporting the hypothesis that thrombin-mediated protease activated receptor-1 receptor activation may link thrombin generation and inflammatory markers measured during cardiac surgery. Although it may be compelling to pursue the trends toward reduced perioperative interleukin 6 and 10 levels we demonstrated with

Table 3. Postoperative Coagulation Profiles by Group

	Baseline	After Heparin Reversal					
	ACT	TAT	ACT	Anti-Xa	ECT	APTT	TBT
Group B							
Median	82	31.6	81	0.04	33.3	27.1	11:00
Range	72–97	28.5–42.1	68–115	0–0.11	28.3–38.6	20.3 to > 150	3 to > 30
Group H&B							
Median	79	52.2*	152*†	0.16*	55.8*	> 150*	18:00
Range	73–90	47.2–60.5	102–161	0–0.22	34.1 to > 150	36.1 to > 150	4 to > 30
Group H							
Median	76	53*	108†	0.18*	37.7	45	> 30:00
Range	67–88	42.4–63.4	93–114	0.12–0.23	31.1–57.1	28.3 to > 150	6 to > 30

Values are expressed as median and range and compared with Wilcoxon rank sum tests; the tail bleeding times, ECT, and APTT values were truncated, and chi-squared analysis of percentages of values abnormally elevated were performed.

* Significantly greater than group B ($P < 0.05$). † Significantly prolonged compared to baseline ACT ($P < 0.05$).

ACT = activated clotting time (in seconds); anti-Xa = anti–factor Xa activity (in units per milliliter); APTT = activated partial thromboplastin time (values truncated at 150 s); ECT = ecarin clotting time (values truncated at 150 s); group B = anticoagulation with high-dose bivalirudin; group H = anticoagulation with standard-dose heparin; group H&B = anticoagulation with standard-dose heparin and low-dose bivalirudin; TAT = thrombin–antithrombin complex levels (in nanograms per milliliter); TBT = tail bleeding time (values truncated at 30 min).

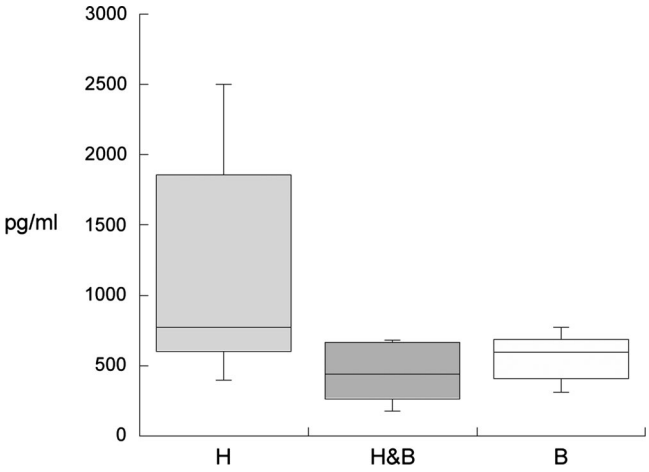

Fig. 3. Change in levels of interleukin 10 by treatment group, measured as the increase from a pre– cardiopulmonary bypass baseline to a time point after a 60-min period of recovery after 60 min of cardiopulmonary bypass. The *box plot* depicts the median and interquartile range; the *whiskers* represent the 10th and 90th percentiles. There is a trend toward an intergroup difference by Wilcoxon rank sum test ($P = 0.057$). B = anticoagulation with high-dose bivalirudin; H = anticoagulation with standard-dose heparin; H&B = anticoagulation with standard-dose heparin and low-dose bivalirudin.

the use of bivalirudin, it remains essential to establish the link between inflammatory markers and outcome in this setting.

One of the limitations of bivalirudin anticoagulation during CPB is the potential for stagnant blood to clot.[15] Theoretically, combining heparin with lower doses of bivalirudin could avoid clotting in stagnant blood and inhibit clot bound thrombin, while reducing dose-dependent plasma levels of bivalirudin.[18,23] Residual bivalirudin after CPB may have been responsible for observed increases in early postoperative blood loss after cardiac surgery with bivalirudin anticoagulation compared with conventional heparin and protamine.[15] It was therefore unexpected that measures of postoperative hemostasis (lower postoperative ACT, ECT, and APTT values) favored the high-dose bivalirudin group. A reasonable dose of 1.25 mg per 100 U protamine sulfate was used for the reversal of heparin anticoagulation in groups H and H&B, and the residual heparin effect we detected was in the range of 0.2 anti–factor Xa units/ml, as previously reported after cardiac surgery with standard protamine dosing.[36] However, residual heparin may not be the explanation. Despite equal amounts of residual heparin in groups H and H&B, only group H&B showed significantly increased ACT, ECT, and APTT values compared with group B (table 3). The ECT, in particular, suggests a residual bivalirudin effect. This seems paradoxical because group H&B received half the bivalirudin dose given to group B, raising the possibility that the rate of elimination of bivalirudin was reduced in group H&B. It is conceivable that bivalirudin consumption that allows clotting of blood in stagnant areas of the CPB circuit[15] was sufficiently prevented by heparin such that the clearance of bivalirudin was reduced. However, there was no significant difference observed between groups H and H&B, and it is not clear whether small intergroup differences in plasma based assays would increase bleeding risk in the absence of a difference in primary hemostasis (table 3), as measured by the *in vivo* tail bleeding time.

Our observation that bivalirudin was ineffective as a sole *in vitro* anticoagulant for our samples underscores the concern surrounding the thrombin catalyzed proteolysis of bivalirudin.[37,38] There is potential for clot formation in critical areas such as partially completed saphenous vein grafts or a clamped internal mammary artery unless close attention is paid to surgical technique, requiring a paradigm shift in clinical practice and thereby introducing a source of serious medical error. This makes our success in suppressing thrombin generation during CPB with a combination of heparin and bivalirudin attractive. However, moving toward clinical application of this strategy would first require establishing a complete dose response of a full range of bivalirudin and heparin doses with regard to thrombin generation, inflammatory markers, and measures of postoperative hemostasis.

Although our model closely resembles current clinical standards with respect to the CPB circuit, there are some limitations. These include the absence of median sternotomy and direct surgery on the heart with reperfusion of ischemic myocardium after cardioplegic arrest. Future study should explore the effect of antifibrinolytic drug use, CPB circuit prime composition, and temperature and use larger sample sizes for measures of postoperative hemostasis to observe more subtle intergroup differences.

In summary, reduction of thrombin generation during experimental CPB can be achieved by adding the direct thrombin inhibitor bivalirudin to conventional dose heparin. However, we were unable to demonstrate that using approximately a half dose of bivalirudin as an adjunct to heparin confers any measurable advantage over full-dose bivalirudin anticoagulation.

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