Effects of Ultrafiltration on Enoxaparin: An In Vitro Analysis

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Abstract: The use of low molecular weight heparins (LMWH) as an anticoagulant in the heparin-resistant patient poses challenges during cardiopulmonary bypass (CPB). The ultrafiltrability of LMWH has not been previously examined. The purpose of this study was to determine the effects of continuous ultrafiltration on the concentration of a LMWH, enoxaparin. An in vitro analysis was performed using fresh whole human blood and an extracorporeal circuit containing four parallel ultrafiltrators and a cardiotomy reservoir with an integrated heat exchanger. Constant conditions included temperature (37°C), flow (0.20 L·min⁻¹), transmembrane pressure (200 mmHg), and hematocrit (25 ± 2%). Samples were collected at the inlet, outlet, and ultrafiltrate line at one and three min for one control trial and again for each of the four hemoconcentrators following the bolus of enoxaparin. Coagulation measurements included a viscoelastic monitor (TEG), activated clotting time (ACT), activated partial thromboplastin time (aPTT), and quantitative analysis utilizing a membrane-based electrode for potentiometric measurement of polyanionic concentrations of enoxaparin. Enoxaparin concentration, from inlet to outlet, increased from 2.95 ± 0.64 to 5.89 ± 0.95 (p < .001) at 1 min and 4.24 ± 0.49 to 7.89 ± 0.606 (p < .001) at 3 min. Kinetic clot activity, as assessed by the TEG index, decreased from −3.8 ± 2.5 vs. −10.5 ± 6.0; (p < .01) pre- to postultrafiltrator samples after 3 min. ACT and aPTT results demonstrated no significant change. In conclusions, this study demonstrates enoxaparin is concentrated with the use of continuous ultrafiltration. Functional coagulation studies also indicate a concentrating effect, primarily via the TEG.

Keywords: ultrafiltration, HIT, low molecular weight heparin, enoxaparin, CPB.

Heparin-induced thrombocytopenia (HIT) is a dose-dependent inflammatory reaction to heparin resulting in immunoglobulin (IgG)-mediated platelet destruction. In classic HIT, exposure to heparin induces the production of an IgG that interacts with a complex of heparin and a component on the platelet surface. This activates and destroys platelets, releasing additional inflammatory substances, which accelerates the process (1). The incidence of HIT occurs in 2–4% of all patients on heparin (2, 3) generally beginning approximately 5 to 10 days after the initiation of heparin therapy. The magnitude of the problem can be appreciated considering the number of patients undergoing cardiac surgery. In 1989, approximately 360,000 patients underwent coronary artery bypass procedures (4).

Patients in need of cardiopulmonary bypass (CPB) and symptomatic for HIT represent a difficult clinical situation pertaining to the method of anticoagulation. The most obvious solution is to prevent heparin exposure by delaying the patient surgery, or delaying surgery until the platelet-aggregating antibodies are undetectable (5). Imperative cases require an alternative method of anticoagulation. Many agents have been utilized to facilitate CPB and include ancrod (6), hirudin (7), and iloprost (8). An additional method of anticoagulation is the use of low molecular-weight heparin (LMWH).

The effect of ultrafiltration on pharmacologic agents utilized during CPB has been an area of much concern. Hirudin and abciximab are both eliminated by high flux membrane ultrafiltrators (9–11). Early theories suggest that heparin is removed during continuous ultrafiltration (12). However, more recent studies indicate a linear increase in the concentration of heparin in blood, as filtrate is removed (13). The purpose of this study was to determine the effects of continuous ultrafiltration on the concentration of a LMWH, enoxaparin.

MATERIALS AND METHODS

The circuit, as depicted in Figure 1, consisted of a hollow fiber ultrafiltrator containing a glycerine-free polysulfone membrane (LifeStream International, Hemochor HPH 1000, Minneapolis, MN) (surface area of 1.06 m² and
priming volume of 70 mL), a nonfiltered venous reservoir with an integrated heat exchanger (Gish Biomedical Inc., Irvine, CA) and polyvinyl chloride tubing. Flow was established through the recirculating system via roller pump (Stockert CAPS, Sorin Biomedical Inc., Irvine, CA). Solutions utilized during this study included two units of fresh (1 day old) CPDA-1 whole human blood purchased from a commercial source (Interstate Blood bank, Memphis, TN), Plasmalyte A, sodium bicarbonate, and enoxaparin (Rhone-Poulenc Rorer Pharmaceuticals Inc., Collegeville, PA).

**Experimental Conditions**

The in vitro circuit was initially primed with Plasmalyte A. Two units of blood were prepared with sodium bicarbonate to adjust the pH to 7.40 ± 0.05 in a separate venous reservoir. The blood was introduced into the circuit and agglomerated with the Plasmalyte A solution, circulating through the four ultrafilters. Baseline hematocrits were 25 ± 2% at the start of sample collection for each ultrafiltrator. Circuit temperature was adjusted to 37 ± 2°C. The flow rate was held constant at 0.20 L-min⁻¹, and a transmembrane pressure (TMP) of 200 mmHg was maintained. Experimental procedures were completed under the manufacturer’s instructions for use.

Following 15 min of equilibrium, three of the ultrafilters were isolated from the circuit. The remaining ultrafiltrator was used to gain an enoxaparin free control. The ultrafiltrate line was opened, vacuum pressure initiated, and baseline samples were drawn. Samples were again collected at 1 and 3 min postbaseline. Ultrafiltrate volume was measured at each time interval. After 3 min of ultrafiltration, the ultrafiltrate line was clamped. The ultrafiltrate volume was added back to the circulating blood to re-establish the initial hematocrit.

A single bolus of enoxaparin (0.30 mg) was administered to create a circulating concentration of 3 units/mL. Data collection for the first treatment ultrafiltrator commenced after 15 min of circulation. Methods were consistent for the four treatment ultrafiltrators and followed the same experimental procedure as identified for the control. Upon completion of each trial, blood was circulated through the untested ultrafilters to prevent stasis during testing periods.

**Sample Collection**

Samples for analysis were collected at the collection points identified in Figure 1. Samples were collected into a syringe at baseline, 1-, and 3-min intervals. These syringes were maintained in a 37°C water bath while awaiting analysis. The samples were recalcified before testing with a 1:17 ratio of blood to 0.2 M calcium chloride to reverse the CPDA anticoagulant. An additional ultrafiltrate and blood sample was collected in EDTA tubes. This blood was centrifuged (4000 rpm, 10 min), and the resulting plasma supernatant was transferred into three 1.5-mL eppendorf tubes, along with the ultrafiltrate sample. These were then stored at −70°C until assayed for LMWH.

**Laboratory Analysis**

Enoxaparin concentration was measured at an outside laboratory (Medtronic Cardiopulmonary, Brooklyn Park, MN). Concentrations of enoxaparin were obtained via membrane-based electrode for potentiometric measurement of polyanions (14). Functional coagulation analyses were performed on the blood samples following the 3-min collection period. The Response (International Technidyne Corp., Edison, NJ) (RES) and the ACT II (Medtronic, Englewood, CO) were used to measure the activated clotting time (ACT). The Bayer Rapid Point Coagulation (Bayer Cardiovascular Diagnostics Inc., Raleigh, NC) (BRP) analyzer as well as the Signature Jr. (International Technidyne Corp., Edison, NJ) (SIG) were used to measure the activated partial thromboplastin time (aPTT). The Thromboelastograph 5000 (Haemoscope Corp., Skokie, IL) (TEG) was used to measure the R time, K time, alpha angle, maximum amplitude, and the TEG index $[T I = \frac{1.792 \times 0.0326 \times (R)^* - 0.189 \times (K)^* + 0.122 \times (M A)^* + 0.076 \times (a)^*}{(\text{celite activated})}]$. Hematocrit and total protein were measured at each sample interval in quadruple and averaged.

**Statistical Analysis**

Results from each of the four treatment hemoconcentrators were combined and entered into spreadsheet format. All values were expressed as mean values ± standard
deviation or percentage change from control. The data were statistically analyzed using SuperANOVA v1.11 for Macintosh. Significant difference ($p \leq .05$) among groups was tested by one-way analysis of variance (ANOVA) followed by Fisher’s Least Protected LSD multiple comparison test.

RESULTS

Figure 2 shows the effect of ultrafiltration on hematocrit, total protein, and enoxaparin concentration. The hematocrit increased by approximately 50% at the 1- and 3-min periods, and the enoxaparin and total protein increased by approximately 100% at the same intervals. Figure 3 numerically represents the concentration of enoxaparin at the inlet, outlet, and ultrafiltrate sample. A significant increase from the inlet to outlet at both 1 and 3 min ($p = .0002, p = .0001$ respectively) was demonstrated.

ThromboelastoGraph

Table 1 shows the results of the parameters measured by the TEG. The R and K time both show an increase from inlet and outlet of the ultrafiltrator at the 1- and 3-min sample periods, with significance achieved at the 3-min interval. The alpha angle declined from pre- to postultrafiltrator at the 1- and 3-min intervals with significance at the 3-min point. There was no significant change pre- to postultrafiltrator in regard to the maximum amplitude. The TEG index decreased from inlet to outlet with significance at the 3-min interval. Figure 5 illustrates a TEG tracing from the 3-min sample, showing the concentrating effect on enoxaparin produced by continuous ultrafiltration.

Activated Partial Thromboplastin Time

The effect of enoxaparin on aPTT is demonstrated in Figure 6a. The SIG and the BRP aPTT values both increased from the control to the treatment period. Figure 6b demonstrates the percent change in aPTT pre- to postultrafiltrator. The SIG shows an increase at 1 min, but a decrease at the 3-min interval. The BRP decreased at the 1-min period, but increased at the 3-min period. Neither machine demonstrated significant differences for any sample.

Activated Clotting Time

Addition of enoxaparin increased the ACT values from the control to the treatment sample. Figure 7a demonstrates this increase, which ranged from 54 to 68% with the ACT II, and a 66 to 88% increase with the RES. Figure 7b demonstrates the change in ACT values measured pre- and posthemoconcentrator. The RES showed a negative change at 1 min and a positive shift at 3 min. The

ACT II showed an increase in ACT values at both the 1- and 3-min intervals.

DISCUSSION

The management of patients with heparin-induced thrombocytopenia presents a dilemma when extracorporeal flow is required. Slocum et al. demonstrated the value of enoxaparin as an alternative to heparin (2). A cross sensitivity exists between enoxaparin and heparin associated antiplatelet antibodies in 34% of patients confirmed with HIT. Enoxaparin may be used safely as a substitute for unfractionated heparin in those patients whose plasma does not aggregate platelets in the presence of enoxaparin. Likewise, CPB has been successfully managed with enoxaparin and other LMWHs (15–17). However, dose regimens and the lack of reversal by protamine sulfate (18) remain areas of uncertainty where further research is required.

LMWH developed commercially have a mean molecular weight that varies from 4000 to 6500 daltons; whereas, standard heparin range from 5000 to 30,000 daltons. Standard heparin (SH) by definition has an antifactor Xa to anti-IIA ratio of 1:1; whereas, LMWHs have an antifactor Xa to anti-IIA ratios of between 4:1 and 2:1 (18). Enox-
aparin specifically has a ratio of 2.7:1 (18). This reduced ability of LMWH to inactivate thrombin when compared to SH is a result of their decreased molecular weight. LMWH also have an increased bioavailability at low doses when compared to SH, which may be a result of their decreased binding to plasma proteins. Clearance of both drugs also differs. Because LMWHs have much lower affinity for heparin-binding proteins than SH, their clearance is independent of dose and plasma concentration; whereas, SH is dose dependent. LMWHs are cleared principally by the renal route, and their biologic half life is increased with renal failure (18).

Drug removal attributable to ultrafiltration is determined by both drug and membrane variables. These variables include electrostatic charge, molecular weight of the drug, protein-binding characteristics, and pore size. The molecular weight does not seem to be, in itself, an important factor in modulating drug sieving. Lau and Kronfol demonstrated that significant correlation was not evident between the molecular weights and the sieving coefficients obtained with polysulfone ultrafiltrators (19). The percentage of protein binding is regarded as the primary variable-limiting drug sieving (19, 20). The sieving coefficients for drugs bound to plasma proteins were substantially increased in ultrafiltration experiments utilizing saline rather than blood (19).

Protein binding seems to be the determining factor for drug concentration in this experiment as well. In reference to Figure 2, the relationship between total protein and enoxaparin concentration is clearly demonstrated. Both components essentially doubled at the 1- and 3-min intervals. In regard to drug concentration, an increase was shown in the concentration of enoxaparin in blood as the filtrate was removed via ultrafiltration. Sixty-two percent of the filtrate samples revealed that no drug was present and the remaining 38% showed only trace amounts.

Table 1. Summary of TEG variables.

<table>
<thead>
<tr>
<th>TEG Parameters</th>
<th>Baseline</th>
<th>1 min Inlet</th>
<th>1 min Outlet</th>
<th>p value</th>
<th>3 min Inlet</th>
<th>3 min Outlet</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>13.8 ± 3.0</td>
<td>11.8 ± 0.3</td>
<td>13.5 ± 1.0</td>
<td>0.3545</td>
<td>13.5 ± 2.7</td>
<td>18.6 ± 4.0</td>
<td>0.0135</td>
</tr>
<tr>
<td>K</td>
<td>6.1 ± 1.7</td>
<td>4.9 ± 0.9</td>
<td>7.8 ± 3.2</td>
<td>0.5012</td>
<td>6.3 ± 2.0</td>
<td>19.3 ± 12.5</td>
<td>0.0074</td>
</tr>
<tr>
<td>MA</td>
<td>45.5 ± 4.0</td>
<td>48.1 ± 3.1</td>
<td>46.3 ± 5.4</td>
<td>0.714</td>
<td>46 ± 5.0</td>
<td>39.3 ± 11.6</td>
<td>0.165</td>
</tr>
<tr>
<td>Angle</td>
<td>54.8 ± 8.3</td>
<td>58.7 ± 2.3</td>
<td>47.2 ± 7.5</td>
<td>0.0057</td>
<td>52.3 ± 8.8</td>
<td>29.3 ± 14.6</td>
<td>0.0029</td>
</tr>
</tbody>
</table>

R, R Time; K, K time; MA, maximum amplitude; Angle, alpha angle.

Figure 4. Thromboelastograph (TEG) Index.

Figure 5. Thromboelastograph (TEG) tracing. A. Baseline sample; B. 3 minute inlet sample; C. 3 minute outlet sample.

Figure 6. A. aPTT% change-control to treatment. BRP, Bayer Rapid Point; SIG, Signature Jr. B. aPTT% change-inlet to outlet. BRP, Bayer Rapid Point; SIG, Signature Jr.
may be attributable to variations within the ultrafiltrators, as well as delineations in flow and transmembrane pressure. The sieving coefficient of enoxaparin was only 0.07 and 0.01 at 1 and 3 min, respectively. A sieving value of one indicates a drug is freely permeable to the membrane, so that enoxaparin was not removed in this experiment.

The TEG was the most sensitive device utilized in the present study to monitor LMWH. This device consistently produced results indicating a concentrating effect of enoxaparin. The decrease in the TEG index pre- to postultrafiltrator, by definition, concludes that an increase in LMWH concentration occurred. This result was consistent for the four treatment hemoconcentrators at both the 1- and 3-min intervals.

Activated partial thromboplastin time has been suggested as an insensitive measurement of LMWH (21). In this study, the SIG produced errors in 60% of the test samples, and the BRP had errors in 35% of the tests. Activated partial thromboplastin time increased from baseline resulting from the addition of the anticoagulant, but the pre- to postultrafiltrator data are inconsistent. The SIG showed concentrating effects at both the 1- and 3-min intervals. The BRP showed a concentrating effect at the 3-min interval and removal of the drug at the 1-min interval.

Activated clotting time is the most frequently performed coagulation test in the cardiac surgical suite. Its usefulness for monitoring LMWH, as with aPTT, has not been demonstrated. A direct correlation between ACT measurements and antifactor Xa activity, a well-accepted method to monitor LMWH, has been reported by Koza (22). However, problems have surfaced when utilizing ACT as the only method of monitoring anticoagulation during CPB (23). In the present study, ACT values increased with the addition of enoxaparin, but inconsistency was again noted. The RES produced results indicating a concentrating effect of the drug. The ACT II points to removal of the drug at the 1-min interval and concentration at the 3-min interval. Both ACT devices produced fewer errors than the aPTT machines. The ACT II displayed 10% error samples, and the RES exhibited zero.

Limitations of this study include the duration of ultrafiltration. The effect of ultrafiltration, whether it be linear of exponential, could be evaluated with additional points. The use of anti-Xa assay may have also been of some use, however, the unavailability of this test to be rapidly performed for clinical use in the operating room dictated this decision. Further studies regarding the effects of continuous ultrafiltration of drug concentration may benefit from altering the flow and TMP, because these factors do affect the rate of ultrafiltration.

In conclusion, this study demonstrates enoxaparin is concentrated with the use of ultrafiltration with minimal drug lost in the ultrafiltrate. Functional coagulation studies also indicate a concentrating effect, primarily demonstrated through thromboelastographic monitoring.

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REFERENCES


