Effects of Bemiparin, Dalteparin, and Unfractionated Heparin on Platelet Interaction With Human Subendothelium Under Flow Conditions

Jose Antonio González-Correa¹, María Monsalud Arrebola², Francisco Mérida³, María Dolores Navas¹, Juan Antonio López-Villodres¹, Felisa Samanes⁴, and José Pedro De La Cruz¹,*

¹Department of Pharmacology and Therapeutics, School of Medicine, University of Málaga, Campus de Teatinos s/n, 29071 Málaga, Spain
²Clinical Laboratory, ¹Infirmary Division, Hospital Universitario Carlos Haya, Málaga, Spain
³Clinical Laboratory, Hospital Comarcal de la Serranía, Ronda, Málaga, Spain

Received January 16, 2008; Accepted March 26, 2008

Abstract. We compared the effects of the low-molecular-weight heparin (LMWH), bemiparin, and dalteparin with unfractionated heparin (UFH) on the platelet subendothelium interaction under flow conditions. All three compounds decreased the percentage of subendothelial matrix covered by platelets, although the effect of LMWH was greater than that of UFH. Subendothelial matrix covered with platelet structures greater than 100 square microns was significantly reduced by all three compounds; the effect of bemiparin tended to be greater than that of the other two heparins.

Keywords: bemiparin, subendothelium, platelet

Low-molecular-weight heparins (LMWH) have been shown to be effective in the prevention and treatment of venous thromboembolism (1), and they are associated with a lower incidence of bleeding than unfractionated heparins. In addition, LMWH has been found to be effective in the secondary prevention of ischemic arterial accidents (acute coronary syndrome, stroke, etc.) (2). In these arterial processes, the interaction of platelets with the vascular subendothelium is an important factor in the pathogenesis and course of ischemia. However, the effects of unfractionated heparin and LMWH on platelet function in ischemia-related disorders are controversial (3, 4).

The aim of this study was to compare the effects of LMWH with a mean molecular weight of 3,600 Da (bemiparin) and 6,500 Da (dalteparin) and unfractionated heparin with a mean molecular weight of 16,000 Da (5) on the platelet-subendothelium interaction under flow conditions.

Whole blood for this in vitro study was obtained from healthy men (mean age 42.8±1.6 years) who had not taken any medication for at least 15 days previously.

Samples were collected with 1.25 or 2.5 units/mL of bemiparin, dalteparin, or UFH (all three from Laboratorios Rovi, Madrid, Spain) and immediately perfused through a perfusion system. A control group consisted of blood samples collected with trisodium citrate 3.8% as an anticoagulant, at a proportion of 1:10 (v/v). Perfusion was done as described by Sakariassen et al. (6) at 37°C during 5 min in a chamber containing a substrate of subendothelium obtained from human umbilical vein endothelial cells in culture. The umbilical cords were obtained after normal labor with informed consent from the mother. Subendothelial matrix preparations were obtained as described by Jaffé et al. (7). The study protocol was approved by the Ethics Committee of the Carlos Haya University Hospital in Málaga, Spain.

For perfusion studies, the blood samples were incubated for 5 min at 37°C. The perfusion chamber was coupled to a peristaltic pump so that the incubated blood circulated for 5 min at a shear rate of 800 s⁻¹ in contact with the subendothelial matrix. After perfusion, each coverslip was fixed with a 0.5% solution of glutaraldehyde and then stained with 0.25% toluidine blue for morphometric analysis. Cell counts were obtained before and after each perfusion to calculate the percentage of platelets retained in the matrices.

In each perfused matrix we calculated the total...
Table 1. Percentages of platelet retention on the subendothelial matrices after 5 min of blood perfusion (shear rate of 800 s\(^{-1}\)) in samples with unfractionated heparin or the low-molecular-weight heparins dalteparin or bemiparin

<table>
<thead>
<tr>
<th></th>
<th>% Platelets retained in matrices</th>
<th>% Matrix covered with platelets</th>
<th>% Matrix covered with structures&gt;100 (\mu m^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (N = 5)</td>
<td>24.83 ± 2.31</td>
<td>15.70 ± 2.25</td>
<td>3.23 ± 0.18</td>
</tr>
<tr>
<td>Unfractionated heparin (N = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.25 U/mL</td>
<td>17.45 ± 0.73*</td>
<td>10.22 ± 0.53*</td>
<td>1.49 ± 0.09*</td>
</tr>
<tr>
<td>2.5 U/mL</td>
<td>12.01 ± 0.99**</td>
<td>8.34 ± 0.87**</td>
<td>0.49 ± 0.06**</td>
</tr>
<tr>
<td>Dalteparin (N = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.25 U/mL</td>
<td>18.56 ± 1.26*</td>
<td>6.72 ± 0.86***</td>
<td>1.29 ± 0.11*</td>
</tr>
<tr>
<td>2.5 U/mL</td>
<td>15.32 ± 1.08**</td>
<td>5.49 ± 0.53***b</td>
<td>0.43 ± 0.05**</td>
</tr>
<tr>
<td>Bemiparin (N = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.25 U/mL</td>
<td>13.30 ± 0.95**</td>
<td>8.90 ± 0.70***</td>
<td>1.20 ± 0.08*</td>
</tr>
<tr>
<td>2.5 U/mL</td>
<td>7.16 ± 0.85**c</td>
<td>5.40 ± 0.11**b</td>
<td>0.35 ± 0.03**</td>
</tr>
</tbody>
</table>

\(*P<0.05, **P<0.001, \) with respect to control samples. \(^aP<0.05\) with respect to 1.25 U/mL unfractionated heparin; \(^bP<0.05\) with respect to 2.5 U/mL unfractionated heparin; \(^cP<0.05\) with respect to 2.5 U/mL unfractionated heparin and dalteparin.

The percentage of subendothelium covered by platelets and the percentages of adhered platelet structures according to their area of adhesion. For each perfused matrix we examined 20 fields. Four matrices were perfused with PBS, fixed, and stained; and these were treated as blank samples. Images were obtained with version 5.0 of the Visilog program (Noesis, Orsay-Cedex, France) and an inverted microscope to which a Sony b/W CCD camera (Sony, Tokyo) was coupled.

All data in the text, tables, and figures are the mean ± S.E.M. of all values for each experiment. One-way analysis of variance (ANOVA) followed by Bonferroni transformation and the paired Student t test were used, and differences were considered significant when \(P<0.05\). All analyses were done with SPSS software, v. 14.0 (SPSS Co., Chicago, IL, USA). The minimum value used to establish statistical significance was \(P<0.05\).

Unfractionated heparin, dalteparin, and bemiparin decreased the percentage of platelets adhered to the matrices after blood perfusion (pre-perfusion platelet count: 294 ± 11 × 10⁶ cells/L) (Table 1). Bemiparin (2.5 U/mL) had the greatest inhibitory effect.

All three compounds decreased the percentage of subendothelial matrix covered by platelets after blood perfusion at a shear rate of 800 s\(^{-1}\) (Table 1), although the effect of LMWH was greater than that of unfractionated heparin (\(P<0.05\)).

The percentage of subendothelial matrix covered with platelet structures with a surface area greater than 100 \(\mu m^2\); was significantly reduced by all three compounds; the effect of bemiparin tended to be greater than that of the other two heparins (Table 1). Figure 1 shows representative pictures of subendothelial matrices after blood perfusion in control assays, with unfractionated heparin, and with dalteparin or bemiparin (2.5 U/mL).

This study shows that LMWH diminished platelet interactions with a human subendothelial matrix under flow conditions. In an annular chamber blood perfusion system with rabbit aorta subendothelium, dalteparin led to greater reductions than unfractionated heparin in platelet interaction with the subendothelial matrix (8). The data from the present study show that bemiparin also inhibited platelet-subendothelium interactions in human tissues.

Bemiparin is a so-called second-generation LMWH (molecular weight less than 5,000 Da) with a mean molecular weight of 3,600 Da, lower than that of the first-generation LMWH dalteparin (6,300 Da) and unfractionated heparin (15,000 Da) (5). Bemiparin has shown beneficial effects in the prophylaxis and treatment of venous thromboembolism (9 – 12). Its anti-\(\mathrm{Xa}\) ratio of 8:1 is higher than the ratio of dalteparin (2 – 3:1) and than that of unfractionated heparin (1:1) (5). Moreover, bemiparin was shown to stimulate the production of tissue factor pathway inhibitor (TFPI) (13).

The concentrations tested in our experiments are based on earlier work that showed these two concentrations inhibited platelet interactions with the rabbit aorta subendothelium after incubation with unfractionated heparin or dalteparin (8). Studies based on classic aggregometric techniques showed that these concentrations did not modify platelet functioning in vitro (8); moreover, bemiparin did not modify collagen-induced platelet aggregation in whole blood (unpublished...
observations). However, under flow conditions in which platelet activation was induced with human subendothelial matrix, the inhibitory effect of the second-generation LMWH bemiparin was greater than that of unfractionated heparin and greater than that of the first-generation LMWH dalteparin.

Two factors have been proposed as the basis for the mechanism underlying this effect (8). Platelet activation leads to the release of factor 4, which partially inhibits the effect of unfractionated heparin (14). In addition, during the process of thrombogenesis factor Xa binds to the platelet membrane, where it helps to generate thrombin, which in turn further activates platelet function (15). Because LMWH has a greater affinity for factor Xa than unfractionated heparin, this may account for the greater effect we saw with LMWH in the flow system used for our assays, as well as for the tendency for bemiparin to have a greater effect than dalteparin, given that the former’s affinity for anti-Xa is 2-fold to 3-fold greater than that of the latter (5).

Thrombin generation from platelet-membrane factor X activation is an important event in the pathogenesis of arterial thrombosis (15). For that reason, the greater inhibition of factor Xa caused by LMWH than UFH could support a theoretical preference for the use of LMWH in the prevention of arterial thrombosis.

In conclusion, the LMWH bemiparin inhibited platelet-subendothelial interactions under flow conditions more than unfractionated heparin did. This inhibition may help account for the effect of LMWH in clinical processes in which platelet interactions with the vascular wall play an important role, as in acute coronary syndrome and ischemic stroke.
Acknowledgments

We thank D. Antonio Pino Blanes for his invaluable technical assistance and K. Shashok for translating parts of the manuscript into English. This research was supported in part by Laboratorios Rovi S.A., Madrid, Spain through an agreement with the University of Malaga (Re. 806/79.1716).

References