MODE OF ACTION OF ENOXAPARIN IN PLASMA

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Abstract. Unfractionated heparin in the extrinsic system has an action on prothrombinase that is insignificant compared to its antithrombin action. In the intrinsic system, unfractionated heparin does have an indirect antithrombin action because its antithrombin activity inhibits the feedback activation of Factor VIII. Most low molecular weight heparins are not different from unfractionated heparin, although their antithrombin action may be slightly higher. Among these, enoxaparin has the highest antithrombin action, due to a relatively high content of very low molecular weight material. In platelet rich plasma, there is an important difference between unfractionated and low molecular weight heparin in that, up to 0.3 U/ml, unfractionated heparin is completely neutralized by activated platelets (300 000 µl/l) whereas low molecular weight heparins are not. Therefore, unfractionated heparin in platelet rich plasma acts only on the lag phase of thrombin production and not on the amount of thrombin produced. Low molecular weight heparins significantly prolong the lag time and inhibit the thrombin peak in platelet rich plasma.

Key words: enoxaparin, low molecular weight heparin, thrombin, prothrombinase, platelet Factor 4, feedback regulation, platelet rich plasma.

Considering the mode of formation of thrombin in plasma, as it is schematically represented in Fig. 1, it can be seen that an anticoagulant product can act according to at least two essentially different pathways. It can act (Fig. 2) directly on thrombin via the catalysis of physiological antiproteases such as antithrombin III (AT III) or heparin cofactor II (HC II) or indirectly by reducing the amount of thrombin generated from prothrombin either by decreasing the prothrombin level, or by acting on the components of the prothrombinase complex, i.e. the activated Factors X and V on a phospholipid surface.

The antagonists of the vitamin K-dependent factors represent a typical example of inhibition of thrombin formation, acting on the Factors II, VII, IX and X. Vitamin K antagonists thus have a typical antiprothrombinase action. Now the question arises: is it necessary to inhibit all of these factors to obtain this inhibition of prothrombinase or only one and, in the latter case, which one? According to a method that has been extensively described elsewhere by these authors (4) the course of prothrombinase activity of the clotting plasmas of patients with anti-vitamin K therapy has been estimated (5). For each vitamin K-dependent factor separately (Factors II, VII, IX and X), the prothrombinase was measured after having supplemented the plasma with the three other purified clotting factors. Figure 3 shows no significant decrease of the prothrombinase activity for the factors VII, IX and X until about 5% of the activity of their normal values. However, in the same figure it can be observed that the prothrombinase activity is directly related to the prothrombin concentration of the plasma. Thus, it appears that prothrombin and consequently thrombin must be the main target for an anticoagulant treatment.

It is known that unfractionated heparin (UFH) inhibits the activated Factors X, IX, XI and thrombin via its AT III cofactor activity. It has been previously demonstrated by the authors (1) that unfractionated heparin (UFH), contrary to the generally accepted view, has hardly any influence on prothrombinase formation triggered via the extrinsic pathway, i.e. by recalcification of plasma in the presence of thromboplastin, as shown in Fig. 4. Its role is essentially that of a catalyst of AT III dependent thrombin inactivation only. Why does the anti-Xa property of unfractionated heparin not play a role in the prothrombinase complex inhibition? As shown in Fig. 3 and as can be seen in Fig. 5, it is necessary to inhibit Factor Xa almost completely, in order to influence thrombin formation via the inhibition of prothrombinase. [This experiment (Fig. 5) was carried out by Lindhout and Pieters (3)]. It demonstrates that when the courses of thrombin and Xa concentration are followed simultaneously in the same thromboplastin-activated plasma, the thrombin peak appears earlier than the Xa peak.
maximum of prothrombin conversion, there is hardly any Factor Xa present. Therefore, it can be concluded that only traces of Xa are necessary to produce a thrombin explosion. Inhibition of Factor Xa therefore has to be important before it starts influencing prothrombin conversion. When in the presence of heparin, coagulation is triggered via the intrinsic pathway (kaolin and phospholipid). An

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**Fig. 1.** A scheme of thrombin formation including the feedback mechanisms.

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**Fig. 2.** Possible modes of inhibition of coagulation by an antithrombotic product.

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**Fig. 3.** Relationship between prothrombinase activity and the concentration of vitamin K-dependent factors.
Mode of action of enoxaparin

Fig. 4. Influence of unfractionated heparin on the thrombin and prothrombinase generation in thromboplastin activated plasma. Control • • heparin (U/ml): ○ - ○ 0.03; ▲ ▲ 0.04; ♦ - ♦ 0.05; ○ ○ 0.1.

Fig. 5. Kinetics of thrombin and Factor Xa generation simultaneously measured in thromboplastin activated plasma in the presence of unfractionated heparin and pentasaccharide. Control: ○ ○ thrombin; ▲ ▲ Xa; • • prothrombinase. Factor Xa generation: heparin (U/ml): △ - △ 0.05; • - ▲ 0.01; pentasaccharide: *25 µg/ml.

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inhibition of the prothrombinase activity and also a prolongation of the lag phase before the apparition of thrombin was observed (1). It can be assumed that this is because the AT III—heparin complex inhibits the earliest traces of thrombin (prolongation of the lag phase) required to convert Factor VIII into Factor VIIIa. Factor VIIIa is required to protect Factor IXa from the AT III—heparin inactivation (Fig. 6). In the absence of Factor VIIIa, Factor IXa (a) can hardly activate Factor X and (b) is readily inhibited by AT III—heparin. Thus, again, it appears that thrombin must be the primary target of an anticoagulant product. Because thrombin enhances its own formation via the mechanism of feedback reactions (Fig. 1), thrombin inhibition causes, secondarily, inhibition of prothrombinase.

Most low molecular weight heparins (LMWHs) tested are not different from UFH, although their antiprothrombinase action may be slightly higher. As shown in Fig. 7, enoxaparin inhibits thrombin in a dose-dependent way. Contrary to most other heparins, it also inhibits the prothrombinase activity in

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**Fig. 6.** A scheme of the action of AT III—heparin complex in plasma.

**Fig. 7.** Influence of enoxaparin on the generation of thrombin in plasma via the extrinsic (curves A, B, C) and intrinsic (curves D, E, F) pathways. A and D: no heparin; B and E: 0.6 μg/ml; C and F: 1.2 μg/ml.
the extrinsic pathway (Fig. 8). Why this difference? enoxaparin is a mixture; and when properly fractionated, two of its major components, fraction A (EMT 966 of relatively high molecular weight) and fraction B (EMT 967 of relatively low molecular weight), have been found to possess different properties (2). As summarized in Table I, fraction A, like UFH (S type heparin), acts primarily on thrombin formed and not on prothrombinase. In contrast, fraction B has no direct effect on thrombin. At high concentration, it inhibits the prothrombinase complex [P type heparin (3)].

In an attempt to investigate the action in a situation nearer to in-vivo circumstances the effect of enoxaparin and its fractions on thrombin generation in platelet rich plasma was studied (Fig. 9). As previously found for UFH (1), enoxaparin was observed to induce a prolongation of the lag phase before the appearance of the thrombin burst. Contrary to UFH, enoxaparin also inhibits the peak of thrombin. It has been shown by the authors (1) that up to 0.05 U/ml of UFH is practically completely inactivated by activated platelets. This means that UFH in platelet rich plasma (PRP) postpones the thrombin burst but

![Mode of action of enoxaparin](image-url)

**Fig. 8.** Thrombin and prothrombinase inhibitions by two batches (white and black symbols) of enoxaparin in thromboplastin activated plasma. The inhibitions are expressed in per cent of the top of the peak of the uninhibited control run in parallel.

![Thrombin generation](image-url)

**Fig. 9.** Influence of enoxaparin and two of its major components on thrombin generation in platelet rich plasma. Control: EMT 966 μg/ml: △ 1.3; EMT 967 μg/ml: ○ 0.7; enoxaparin μg/ml: ■ 4; ▲: EMT 966 1.3 μg/ml + 0.7 μg/ml EMT 967 (recombined enoxaparin).
Table I. Inhibition of extrinsic coagulation by the two major components of enoxaparin. The inhibitions are expressed in per cent of an uninhibited control run in parallel

<table>
<thead>
<tr>
<th>Concentration (µg/ml)</th>
<th>Inhibition (%)</th>
<th>Thrombin</th>
<th>Prothrombinase</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMT 966 0.35</td>
<td>60</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>(Fraction A) 0.5</td>
<td>72</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>EMT 967 5</td>
<td>47</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>(Fraction B) 10</td>
<td>79</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>

hardly inhibits it, while enoxaparin does inhibit the generation of thrombin, most probably because enoxaparin presents a lower susceptibility to platelet Factor 4 (4) released by activated platelets. This may be an example of a general mechanism by which LMWHs that by themselves are inactive but may, in platelet rich plasma, potentiate the action of active heparin components.

In conclusion, enoxaparin is a mixed type heparin which inhibits both thrombin and prothrombinase generation. It is likely that enoxaparin influences the generation of thrombin primarily, by its relatively high molecular weight fraction (S type component) and secondarily by its P type component, i.e. the relatively low molecular weight fraction. Inhibiting thrombin, enoxaparin can also inhibit the feedback mechanism, inducing an inhibition of prothrombinase, possibly reinforced by anti-Factor Xa property of its P type fraction.

REFERENCES