

The anticoagulant mechanism of action of heparin in plasma

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The Anticoagulant Mechanism of Action of Heparin in Plasma

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The process of blood coagulation has been described as a series of enzyme activations and in each step of this sequence, the activated zymogen converts another zymogen to an active protease. At present, we know that this series of linked proteolytic reactions in fact comprise a series of interlinked positive and negative feedback loops. The generation of activated clotting factors at a significant rate requires the formation of a complex between a zymogen, a cofactor and an enzyme on a surface (phospholipid membranes). The positive feedback loops function in the generation of cofactors and procoagulant surfaces (activated blood platelets) by products generated at later stages of the coagulation sequence. The negative feedback loops cause the inactivation of the multi-molecular complex by destruction of the cofactor.

Most of our knowledge about the biochemistry of these reactions emerged from *in vitro* studies in purified systems. At present, little information is available to what extent each of these reactions contribute in the overall reaction rate of the blood coagulation sequence.

Because heparin and heparin fractions, by virtue of their anti-factor Xa and anti-thrombin activity, might act on each of the zymogen-serine protease and procofactor-cofactor transitions, it is extremely difficult to assess the relative importance of the anti-factor Xa and anti-thrombin activity to the overall rate of thrombin generation in plasma. As a result, standardization of heparin and the laboratory control of the clinical use of this anti-thrombotic drug are highly debatable issues and presently remain unresolved.

The aim of our current studies is to examine separately in clotting plasma the contributions of the heparin-dependent factor XA inhibition and thrombin inhibition in the generation of thrombin. To this end, we monitored the generation of activated clotting factors in plasma activated via the intrinsic or extrinsic pathway in the presence of heparin and heparin fragments. Because the currently used amidolytic assays for activated clotting factors are insufficient to measure each of the ser-

ine proteases or cofactors separately in plasma, we developed highly sensitive and specific bioassays. Because of the large numbers of assays and their handling complexity it was highly desirable to have a laboratory workstation to do the job. At present, the following activated blood coagulation factors can be measured utilizing the BIOMEK 1000 automated workstation: factor VIIIa, factor Va, factor IXa and factor Xa. All these factors can be accurately measured in the picomolar range.

The generation of factor Va, factor Xa, prothrombinase and thrombin in thromboplastin-activated plasma has been analyzed in the absence and presence of heparin and heparin fragments (low molecular weight heparins). Our findings together with those obtained with the synthetic pentasaccharide heparin and standard heparin strongly suggest that the anti-factor Xa activity of heparin contributed little in its anticoagulant activity. The inhibition of factor Va generation, dependent on the heparin anti-thrombin activity only, is of prime importance to the inhibition of thrombin generation in plasma. The inhibition of thrombin generation by low molecular weight heparins was comparable with that of standard heparin on the basis of their respective anti-thrombin specific activities, but not on basis of their anti-factor Xa activities.

In platelet rich plasma, low molecular weight heparin has a relatively high anti-thrombin specific activity when compared with standard heparin in a range up to 0.2 USP units/ml. In contrast to standard heparin, low molecular weight heparin is much less susceptible for neutralization by platelet factor 4.

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