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There are three main classes of antithrombotic drugs: Platelet inhibitors, oral anticoagulants, and drugs such as heparin that inhibit activated clotting factors. Oral anticoagulants and heparins are roughly equally effective in preventing venous thrombosis. However, these two families of drugs are essentially different in their mode of action. They each have numerous properties in addition to their anticoagulant action, but for neither of the two has it been demonstrated that it is indeed the anticoagulant action that is responsible for the antithrombotic effect. Yet, the anticoagulant action is the only action that they have in common. Therefore we consider it more likely than not that the anticoagulant property bears some direct relationship to the therapeutic effect. Despite active research on platelet aggregation inhibitors for over 25 years, it is now generally acknowledged that these substances make less effective antithrombotic agents than anticoagulants do.[1] It must even be considered a possibility that antiplatelet drugs act via an activation on platelet-induced thrombin formation.[2]

We will therefore discuss the mode of action of heparins and other thrombin scavengers in terms of inhibition of the coagulation process.

Neither oral anticoagulants nor heparins are ideal drugs. Oral anticoagulant treatment requires laboratory control and constant expert supervision. Heparin needs frequent administration by injection and side effects are common. Both drugs have a relatively narrow therapeutic window, that is, the gap between the effective dose and the dose that will cause bleeding, is not very wide. This explains why at this moment much active research is spent in finding more efficient antithrombotics.

In this article we will discuss those drugs, such as heparin, that are known to scavenge active clotting factors. Within this group, there are drugs with quite dissimilar actions. Pentasaccharide, for instance, only enhances antithrombin III (AT III)-mediated Factor Xa inactivation, whereas hirudin is a specific thrombin inhibitor. Yet this group of drugs also has many features in common, so many, indeed, that we may consider them to belong to one "superfamily" that we will call "scavengins" (Table 1). As a typical example we will first discuss the mode of action of heparin. This requires a short introduction on the reaction mechanism of blood coagulation.

THE MECHANISM OF THROMBIN FORMATION

Three fundamental processes govern the generation of thrombin in platelet-poor plasma: (1) sequential activation of proteases, (2) enhancement of the efficiency of a protease by its complexing to a protein cofactor at a phospholipid surface, and (3) activation of the protein cofactors by thrombin, the end product. In platelet-rich plasma one more process should be taken into account: the platelet membrane flip-flop.

The sequence of protease activations differs according to the way in which coagulation is triggered. When the reaction is started with excess tissue thromboplastin, this is done through the extrinsic pathway: Factor VII→ Factor X→ Factor II. In the intrinsic pathway the reactions can be summarized as: Contact Factors→ Factor IX→ Factor X→ Factor II. In that case the contact factors are activated by kaolin, ellagic acid, or other negatively charged "foreign" surfaces. Under pathophysiologic conditions, the triggering of coagulation by small amounts of tissue factor is probably important. In that case the indirect activation of Factor X via the action of Factor VII on Factor IX has to be taken into account. This pathway we called the Josso loop: Factor VII→ Factor IX→ Factor X→ Factor II.[3,4]

Neither activated Factor VII nor Factor IXa nor

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TABLE 1. Scavengins: Drugs That Act Directly or Indirectly by Inactivating Clotting Proteases

Name	*Abbreviation*	*Cofactor*
Unfractionated heparin	UFH	AT III
Low MW heparin	LMWH	AT III
Pentasaccharide	Penta	AT III
Pentosan polysulfate	PS	AT III + HC II
Stichopus Japonicus mucopolysaccharide	SJAMP	AT III + HC II
Dermatan sulfate	DS	HC II
Lactobionic acid	LBA	HC II
Hirudin	Hir	None

Factor Xa are very efficient enzymes on their own. All three need to be adsorbed on a procoagulant lipid surface next to a protein cofactor. In order to be procoagulant, a phospholipid usually has to contain a certain amount of phosphatidylserine. The cofactors are specific to each of the clotting factors: Factor VII(a) needs the protein moiety of tissue factor, Factor IXa needs Factor VIIIa and Factor Xa needs Factor Va (see Tans et al[5] for a review).

Factor Va and Factor VIIIa do not occur as such in plasma but arise from their precursors (Factor V and Factor VIII) by the action of thrombin.[6–8] Factor Xa can also exert its function under certain in vitro conditions, but this probably does not play a role in clotting plasma.[9] Thrombin is the end product of the reaction sequence. Its action on Factors V and VIII is therefore a prime example of positive feedback.

Phosphatidylcholine (PC)-containing surfaces are not readily available to the blood clotting factors in native plasma because this phospholipid occurs practically only on the inner side of cell membranes. That is why intact cells are usually not procoagulant. Thrombocytes, however, are an exception. When they are triggered by thrombin, a transbilayer phospholipid movement ("flip-flop") in their cell membrane causes them to expose PC at the outside of the intact cell (see Bevers et al[10] for a review). At the same time, they undergo shape change and produce PC containing procoagulant microvesicles. This constitutes a third positive feedback mechanism by thrombin.[11]

The essential reactions governing thrombin formation are summarized in Figure 1.

THE THROMBIN GENERATION CURVE

In Figure 2 we show the features of a thrombin generation curve. The compulsory thrombin-dependent activation of Factors V and VIII and, as the case may be, of platelets (processes c and d) requires a more or less pronounced lag time. During this lag time slow thrombin generation by an as yet unknown mechanism causes a small amount of thrombin to arise. One can imagine that minute amounts of thrombin can be formed without the cofactors present or that occasional molecules of the cofactors are activated via alternative pathways. Anyhow, the presence of a small amount of thrombin during a certain time is necessary for the full activation of the system and for explosive further thrombin formation.

Of the three feedback mechanisms, that of Factor V is associated with the smallest lag time, that of Factor VIII takes more time, and that of platelets still more. Therefore the lag times in the extrinsic system are short, in the intrinsic system they are longer, and in platelet-rich plasma they are the longest.

Clotting times are essentially experimental determinations of this lag time, clotting ensuing invariably when about 20 nM of thrombin has formed. The classic clotting tests do not measure the amount of thrombin formed. In pathophysiologic situations very probably both the lag

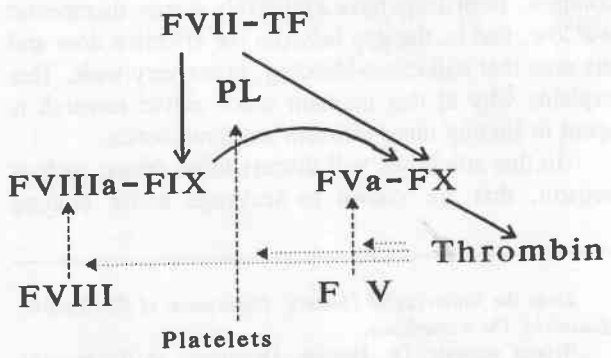

FIG. 1. A scheme of blood coagulation. Solid arrows indicate sequential activations, dashed arrows show chemical conversions, dotted arrows, feedback activations. PL: phospholipid; TF: tissue factor.

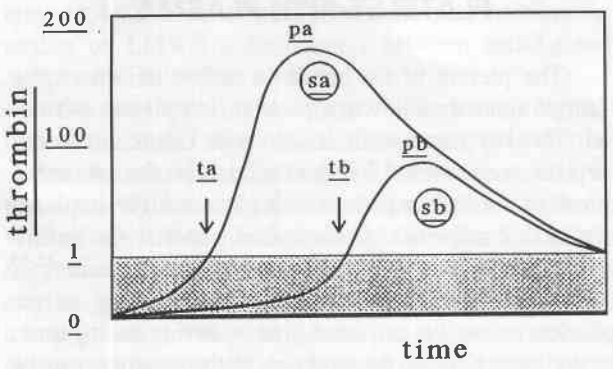

FIG. 2. The thrombin generation curve. The ordinate is nonlinear in that the range of 0 to 1 (gray zone) has been extended in order to visualize the generation of small amounts of thrombin during the lag phase. a: normal curve; b: curve in the presence of a scavengin; t: lag time; p: peak amount; s: surface under the curve.

time and the amount of thrombin formed in situ at the site of a vessel injury determine the thrombotic or hemostatic reaction. This may be the reason why there are so often discrepancies between the prolongation of a clotting time by a scavengin and its antithrombotic action. Often, no influence on clotting time tests is interpreted as no influence on thrombin generation. From this error to the conclusion that there exist scavengins that do not influence blood coagulation is a small, and often encountered, but illogical step. In fact, every scavengin that we tested thus far (essentially those mentioned in Table 1) markedly influenced the amount of thrombin formed. That is why we are developing a test that responds to the area under the thrombin generation curve rather than to its lag time.

MODE OF ACTION OF HEPARIN

Heparin influences thrombin generation in two ways, both of which are due to its enhancing the thrombin-inactivation rate. There is the effect on the bulk amount of active thrombin present at any moment and the effect on the lag time that is caused by the diminution of the minute amounts of thrombin necessary for feedback activation. Although both effects are caused by the scavenging of thrombin, they are not invariably coupled. Heparin will, for instance, hardly influence the lag time of the extrinsic system (thromboplastin time), but it will exert a marked influence of the lag time of the intrinsic system (activated partial thromboplastin time) and on the clotting time of platelet-rich plasma. The reason for this is that the intricate kinetics of clotting factor activation are not the result of a simple relationship between the amount of thrombin present during the lag time and the formation of prothrombinase (see later).

Heparin has been shown to have, in the extrinsic system, only an insignificant effect on prothrombinase (that is, Factor Xa-Va-phospholipid) activity, despite its definite anti-Factor Xa activity.[2,12,13] This may be explained by the relative excess of Factor Xa in clotting plasma. The maximal velocity of thrombin generation that we observed is about 300 nM · min^{-1}. The turnover number of a complete prothrombinase moiety (Factor Xa, Factor Va, phospholipid) is on the order of 2000 min^{-1}.[14] This allows us to estimate the maximum prothrombinase plasma concentration as 0.15 nM. The plasma concentrations of both Factor V (25 nM) and Factor X (200 nM) are much higher than this. This means that only a small fraction of Factor V has to be activated to stimulate the system. This may explain the short lag times in the extrinsic system. It also explains why prothrombinase generation is virtually insensitive to Factor Xa inhibition. Indeed, Pieters et al[15] showed that it is possible to inhibit Factor Xa generation so as to have no measurable Factor Xa present (<0.5 nM) and yet to obtain full prothrombinase activity, provided that sufficient Factor Va (1 nM) was available.[15]

In the intrinsic system we cannot for the moment make an educated guess of the amount of tenase necessary to trigger explosive thrombin formation, but the qualitative data are clear. The rate-limiting step is the thrombin-dependent activation of Factor VIII. Heparin inhibits because it diminishes the thrombin level that activates Factor VIII during the lag time. In any case the main action of heparin is via the inhibition of thrombin and thrombin-mediated feedback mechanisms.[2,12,13,16]

LOW MOLECULAR WEIGHT HEPARINS

Apart from pentasaccharide and related ultra low molecular weight heparins (ULMWH), all heparins act essentially like unfractionated heparin (UFH).[2,12] The low molecular weight heparins (LMWH) that are in medical use at the moment do not belong to the ULMWH group. In platelet-poor plasma the action on the biochemical mechanism of thrombin formation is essentially identical for LMWH and UFH. We therefore group them together as S heparins (they behave as standard heparin). The ULMWH group we called P heparin (for pentasaccharide).[2]

Even though the anti-Xa action of LMW S heparins is higher than their anti-IIa action (taking normal heparin as the reference material),[17–22] this does not result in their inhibiting at the level of prothrombinase (where Factor

Xa is the active enzyme) because of the large Factor X reserve in plasma, as already explained. This is why a heparin concentration expressed in anti-Xa units does not give a realistic picture of its anticoagulative power.

Pentasaccharide and other P-type heparins pose a conceptual problem to some people because they do not act on thrombin and they still are antithrombotic. This is in apparent contradiction with the concept of thrombin inhibition as being crucial to the antithrombotic action. It should be remembered, however, that any anti-Xa action eventually results in less thrombin being formed. We have demonstrated that pentasaccharide acts on free Factor Xa.[22] In this case, where no anti-IIa action is present, the inhibition of Factor Xa can be so important that it becomes rate limiting. With any heparin that does have an anti-IIa action, this action always overrules the anti-Xa action. At the moment, we are trying to find, with the aid of precisely defined heparin fractions, at what molecular weight the P-S transition takes place. *Obiter dictum:* The fact that pentasaccharide does not influence the anti-IIa action of AT III does pose a theoretical problem. If pentasaccharide induces a conformational change in AT III that makes it more active toward Factor Xa, it is difficult to conceive why it should not also have a certain action on the AT III-thrombin interaction. Evidently, the mechanism is more complicated than commonly thought. That the anti-Xa action of pentasaccharide does not per se constitute its antithrombotic potency is easily seen from the fact that one needs to give about 10 times more anti-Xa units of pentasaccharide compared with UFH to obtain a comparable antithrombotic effect.[23,24]

NONHEPARIN SCAVENGINS

The mode of action of the nonheparin scavengins is again principally the same as that for S heparins: inhibition of thrombin and thrombin-mediated feedback. This is without doubt the case for hirudin.[25,26] Also, the heparin cofactor II (HC II)-dependent scavengins such as dermatan sulfate and lactobionic acid act in this way (see Béguin et al in this issue of *Seminars*).[27,28] This does not exclude the possibility that they have additional actions. Pentosan polysulfate, for instance, that is partly HC II and partly AT III dependent also inhibits directly the thrombin-dependent activation of Factor VIII, and to a lesser extent that of Factor V.[12,29–31]

It thus seems that the global mode of action of scavengins in platelet-poor plasma can be summarized as: *inhibition of thrombin and thrombin-dependent feedback* reactions noting that for the P heparins this is an indirect effect mediated by their direct anti-Xa action.

PLATELET-RICH PLASMA

The picture of the mode of action of scavengins changes dramatically when platelet-rich plasma is studied. The key experiment is shown in Figure 3. To our surprise, we see that UFH does not inhibit the *amount* of thrombin formed in platelet-rich plasma.[2] The explanation is that activated platelets shed material (by definition, platelet factor 4 [PF4]) that neutralizes heparin.[32,33] UFH therefore only has an influence so long as the platelets are not yet activated, that is, during the lag time. In the beginning of the experiment the medium can be regarded as platelet-poor plasma in which inert platelets are suspended. The mechanisms in platelet-poor plasma operate so that only limited amounts of thrombin are available so that it takes a relatively long time before the platelets become activated. Once they are activated, they neutralize the heparin present and thrombin generation proceeds essentially undisturbed.

The experiment once more emphasizes the ease with which platelet activation products neutralize heparins and hence the importance of collecting blood on platelet inhibitors when reliable information on the heparin level in a patient is to be obtained. Routine venipuncture activates platelets so as to neutralize 0.22 ± 0.11 U/ml of UFH.[34] Eight of ten pharmacokinetic studies on heparins can be disregarded because this simple fact has been overlooked. LMWH are less readily neutralized than UFH, maybe because they possess less PF4 binding sites per molecule or because they contain much, otherwise inert, PF4 binding material or because they are administered in higher molar concentrations so that they more easily titrate the available PF4. Therefore, LMWH will inhibit thrombin generation in platelet-rich plasma and probably in vivo, too (Fig. 2).[35] The preferential neutralization of higher molecular weight LMWH by PF4

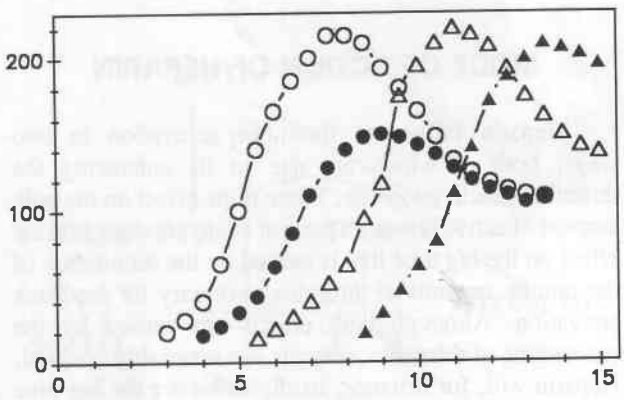

FIG. 3. The influence of heparins on thrombin generation in platelet-rich plasma. ○: control; △ : unfractionated heparin 0.1 U/ml (~0.5 μg/ml); ▲ : unfractionated heparin 0.2 U/ml (~1.0 μg/ml); ●: Fraxiparine (5 μg/ml).

may explain, at least partly, why in pharmacokinetic studies on LMWH a discrepancy between anti-Xa and anti-IIa action is often observed.

One may ask why heparin in vivo has an antithrombotic action if this action is linked to thrombin inhibition and if the amount of thrombin is not decreased in platelet-rich plasma, a situation obviously closer to the in vivo situation than platelet-poor plasma is. A possible suggestion for an answer is to be found in the fact that, in vivo, heparin acts in flowing blood. Due to the flow, a retardation of thrombin generation will automatically mean more mixing and dilution and hence lower thrombin concentrations, even if the net amount of thrombin generated is not diminished.

CONCLUSIONS

1. The main mode of action of scavengins is to decrease the amount of active thrombin and to impair the thrombin-dependent positive feedback on thrombin generation.

2. Anti-Factor Xa action is of no importance in either UFH or LMWH now available to the clinician (S heparins). In dermatan sulfate and other HC II-dependent scavengins, it plays no role at all and obviously not in hirudin either. It comes into play in pentasaccharide and some other very low molecular weight heparins (P heparins).

3. A realistic estimate of the potency of a scavengin can only be made if we take into account its susceptibility to being neutralized by products from activated platelets.

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