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# A rational approach to heparins

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**Abstract.** *It is shown why the current practice of expressing heparin activity in International Units is not an adequate way to deal with the situation that arises after injection of low molecular weight heparins. An alternative approach is proposed. It is taken into account that the fraction of a low molecular heparin that binds with high affinity to antithrombin III contains two fundamentally different components : the material above the critical chainlength of 17 sugar units that has both anti-factor Xa activity and antithrombin activity (ACLM) and the material below that length with anti-factor Xa activity only (BCLM). It is shown how the levels of ACLM and BCLM in a plasma sample can be determined and expressed in μg/ml.*

**Une approche rationnelle des héparines**

**Résumé.** *Il est démontré que la pratique habituelle d'exprimer l'activité de l'héparine en Unités Internationales n'est pas une façon adéquate de répondre à la situation qui fait suite à l'injection des héparines de bas poids moléculaire. Une autre méthode est proposée. Elle tient compte du fait que la fraction d'une héparine de bas poids moléculaire qui se lie avec une forte affinité à l'antithrombine III contient deux composants radicalement différents : celui dont la longueur de la chaîne est supérieure à 17 unités monosaccharidiques a une activité anti-facteur Xa et antithrombine et celui dont la longueur est inférieure et qui ne possède qu'une activité anti-facteur Xa. On démontre comment ces deux activités peuvent être mesurées dans un échantillon de plasma et exprimées en μg/ml.*

**Key words :** *(Low molecular weight) heparin — Antithrombin activity — Antithrombin III — Anti-factor Xa activity — Pentasaccharide*

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It is not necessary for an efficient treatment to be based on rational reasoning, nor will a therapy automatically follow from sufficient scientific knowledge of a disease. Psychotherapy has its therapeutic successes even though Popper explains us that no hard science is involved and no cure for AIDS has as yet evolved despite our detailed knowledge of its cause. That is what makes the difference between the art of the doctor and the science of medicine.

Yet there is a supreme reason for trying to push medicine as far as possible in the direction of the natural sciences : reproducibility. The failure of an ununderstood therapy may be due to any reason, from fundamental misconception (e.g. homeopathy) to stochastic variation, and trial and error is the only way to improvement. The failure of a therapy with a sound scientific basis is open to analysis and allows a design for amelioriation.

For many years and up to the present moment the

effectiveness of heparin therapy has been recognised but not understood. A role of the anticoagulant action of heparin is our savest guess. Indeed the evidence for a central role of thrombin formation in the genesis of thrombosis is becoming overwhelming. If not recent research had shown how intricately platelet physiology is related to thrombin formation, it would hardly have been elegant to stress this point in an article dedicated to a scientist who has so ardently devoted his career to the blood platelet.

If we want to test the hypothesis that the anticoagulant action of heparin is the therapeutic one, then correct quantitation of this effect is the first requirement. This is a field fraught with difficulties, to some of which we think to have found rational solutions recently.

As long as unfractionated heparin was the unique species available the quantitation of its effects was relatively straightforward because in the different preparations the ratio of the different activities was fairly constant. Hence, if one measured e.g. an anti-factor Xa (aXa) dependent ex vivo test, it would correlate with any antithrombin (aIIa) dependent in vivoπP effect, because there was a fixed relation between the two activities. With the advent of low molecular heparins however, it became possible to vary the aXa- and aIIa activities independently in different heparin preparations. The convenient covariation of the two different anticoagulant activities was lost. Yet quantification continued to be carried out in terms of one biological effect only, often the anti-factor Xa activity, alternatively the aPTT, the heptest, the thrombin time etc. The carry over, onto low molecular weight heparins, of habits that were satisfying for unfractionated heparins, has been at the basis of much confusion. At this moment, based on the insights from recent research, it seems possible to design a more rational approach.

## The biochemical basis of heparin action

The biochemical basis of heparin action is the fact that it catalyzes the inactivation of proteases by inhibitors available in the plasma.

As an example we take the inactivation of factor Xa (F.Xa) by antithrombin III (AT III).

$$\text{F.Xa} + \text{AT III} \xrightarrow{k} \text{inactive product}$$

Usually there is much more AT III than FXa so that the final concentration of this reactant will not be very different from the starting concentration[1]. Therefore the reaction will obey pseudo first order kinetics i.e. the reaction velocity (v) is proportional to the initial concentration of AT III and the temporal concentration of F.Xa. Elementary reaction kinetics learns that in this situation F.Xa activity will disappear logarithmically. That is to say that its inactivation is characterized by a half-life time ($t_{1/2}$) that is inversely proportional to the reaction constant (k) and to the initial concentration of AT III. The AT III concentration multiplied by k governs the rate of decay of enzyme activity. It therefore is often refered to as the decay constant: $k_{dec}$ . The dimension of $k_{dec}$ is the inverse minute, because it is proportional to the inverse of the half-life time measured in minutes[2].

In order to be active in catalysing AT III mediated reactions, heparin has to contain the specific pentasaccharide through which the binding to AT III is mediated [6]. Additionally for catalysing the inhibition of thrombin it is necessary that the chainlength is not shorter than 18 monosaccharide units [1, 2, 7-9, 14, 18, 19]. In every heparin preparation one can therefore distinguish the high affinity material (HAM), that contains the pentasaccharide versus the low affinity material (LAM) that does not. Within the HAM fraction one can again distinguish between the material above the critical chainlength (ACLM) that is able to act on both thrombin and factor Xa inactivation, and the material below the critical chainlength (BCLM) that only acts on factor Xa [5, 12]. One can determine the *specific anti-factor Xa activity of a heparin*, which is the increase of $k_{dec}$ of factor Xa brought about *in plasma* by 1 µg/ml reaction medium of the heparin in question. One can also determine the specific antithrombin activity of a heparin, which, analogously, is the increase of the $k_{dec}$ of thrombin *in plasma* brought about by 1 µg/ml of the heparin [12]. Because $k_{dec}$ is proportional to the concentration of AT III it should be normalised to plasma containing 1 µM of AT III by dividing it through the actual AT III concentration of the plasma used.

The specific anti-factor Xa activity is caused by the HAM molecules, the specific antithrombin activity by the ACLM molecules. Later we will discuss what else influences these constants.

Studying a large series of different heparins, we found that the specific activities of all ACLM prepara-

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[1] This holds at any given instant during the clotting of blood when free F.Xa (and thrombin) is available. The peak F.Xa concentration in clotting plasma is ~20 nM, that of thrombin ~300 nM, whereas AT III is present at concentrations > 2 µM. It does not mean that the concentration of AT III remains constant during the whole of the clotting process. In serum the AT III concentration will equal roughly the AT III concentration of plasma minus the prothrombin concentration of plasma

[2] The relevant formulas are
$C_t=C_o.\ e^{k_{dec}.t}$ or $\text{Ln}\ C_t=\text{Ln}\ C_o - k_{dec}\ t.\ k_{dec} = k.\ [\text{AT III}]$.
From this it follows that the half-life time $t_{1/2} = \text{Ln}\ 2/k_{dec}$. The first formula can also be written as $C_t=C_o.\ (1/2)^{t/t_{1/2}}$, which immediately shows the relation between half-life time and semilogarithmic decay

tions, obtained from different types of LMWH, vary within narrow limits, both with regard to the aIIa and to the aXa activity (e.g.the specific aIIa in the ACLM fractions of ten different LMWH preparations was found to be 14.1 ± 2.7 $min^{-1}$ /(μg/ml)/μM AT III (calculated from data in [11]). The same holds for the specific aXa activity of the BCLM preparations. This means that the differences between different heparins are primarily due to their HAM, ACLM and BCLM content.

It is interesting to see that ACLM is more potent than BCLM is. In a mixture ACLM will as a rule overshadow the activities of BCLM, unless the proportion of BCLM becomes very large. This is the explanation of the observation that most LMWHs, like UFH inhibit free thrombin in clotting blood primarily by scavenging thrombin already formed, the inhibition of prothrombin conversion being much less important [10]. This group of heparins we called S (standard UFH-like) heparins. Only those preparations that do not or hardly contain ACLM, will act via inhibition of prothrombin conversion. We called them P (pentasaccharide-like) heparins [10].

Although in vitro BCLM is much less potent than ACLM, it may gain in importance in vivo. In the first place platelet factor 4 (PF4), released from activated platelets can completely neutralise ACLM but will not inactivate BCLM as thoroughly [14]. In the second place the biological half-life time in the circulation is shorter the longer the chainlength is. Therefore BCLM survives longer in the circulation than ACLM. After injection there is a "heparin fractionation in vivo that favours the persistence of BCLM molecules [12].

## How to determine ACLM and BCLM levels in plasma

In plasma the aXa activity and the antithrombin activity can be readily determined. If the specific activities of the heparin injected are known one can readily obtain the ACLM level by deviding the increase of the $k_{dec}$ of thrombin caused by the heparin through the specific antithrombin activity of that heparin [12]. One should be aware of the fact that in this way one measures the amount of ACLM molecules active in the plasma, i.e. bound to AT III. As we will see later this is not automatically the same as the concentration of those molecules in the plasma. Here it suffices to say that it is anyhow the *active* concentration. The level of all active HAM molecules is obtained by deviding the increase of the $k_{dec}$ of factor Xa by the specific anti-factor Xa activity. The level of BCLM can then be obtained by subtraction of the HAM level and the ACLM level [12]. Preliminary results show that, after subcutaneous injection, ACLM disappears with a higher velocity than BCLM. This alone makes it clear that the pharmacology of a LMWH cannot be treated as the pharmacology of one single chemical species and that it is necessary to at least recognise the two species mentioned. This however raises the question : is this sufficient or are there additional complications ?

## Is it necessary to reckon with other effects ?

*Other clotting factors and inhibitors*

In plasma there are more clotting proteases than only thrombin and factor Xa and there is at least one more heparin-dependent inhibitor. Yet, in our opinion, in a first attempt to rationalisation, we may probably restrict ourselves to two variables that we discussed above.

Although the factors of the contact system are not insensitive to heparin action [17], the effect of the normal doses of heparin on this system are negligible and can for all practical purposes be disregarded if we restrict ourselves to the effects of prophylactic and therapeutic heparin concentrations [15].

Factor IXa is stable in plasma until heparins are added [16]. This anti-factor IXa effect contributes significantly to the anticoagulant effect of heparin in the intrinsic system [4]. We did not yet investigate LMWHs in this respect. The available data [13] suggest that the Mw dependency of the aIXa action is identical to that of the aIIa activity. If this is true, then there is no need for a separate consideration of the aIXa effect if one wants to quantitate the heparin effect because it will covariate, and therefore be adequately rendered by the antithrombin effect.

Heparin cofactor II (HC II) is an inhibitor of thrombin only, that is stimulated by heparin. Tenfold higher concentrations of heparin are however needed for this factor than for AT III [20]. Again the effect of this factor may be disregarded unless very high doses of heparin are given (e.g. artificial circulation).

*Heparin binding proteins*

In plasma AT III is not the only heparin binding protein (HBP). Histidine rich glycoprotein and many other plasma proteins compete with AT III for circulating heparin. This explains why the effect of heparin is proportional to the concentration of AT III, even though AT III (~ 2 μM) is present in large excess over the highest possible concentration of heparin (1 U/ml ≈ 0.3 μM). In a purified system this would lead to practically complete binding of all heparin and adding more AT III would not appreciably change the situation. In plasma, with much competing proteins present, increasing (decreasing) the concentration of AT III will proportionally ameliorate (worsen)

its position in the competition.

The binding to the other proteins is however not via the specific pentasaccharide of the heparin. This means that it must be possible to set free anticoagulant heparin from heparin binding proteins by adding non-anticoagulant, low affinity heparin (LAM). A rough estimate tells that about 40 % of the potentially active heparin indeed binds to AT III. The active concentration of HAM, calculated by the procedures mentioned above is therefore 40 % of the real concentration.

*The effect of platelets*

Upon activation platelets release PF4, a protein that binds strongly to heparins. The maximal amount of PF4 released in normal platelet rich plasma (PRP) can neutralize some 0.4 U/ml of unfractionated heparin [3]. All ACLM can be readily inactivated by PF4 but BCLM binds with significantly lower affinity and cannot be inhibited completely. LMWHs therefore retain some activity, and especially BCLM, i.e. anti-factor Xa activity in the presence of activated platelets. What difference this makes as to their efficiency as antithrombotics is anybody's guess. One can imagine that in and around platelet rich thrombi, where the PF4 concentrations must be enormous, a LMWH that retains some of its activity should be more efficient than UFH. If this is true then the Choay pentasaccharide should be a more efficient antithrombotic than could be predicted from its anticoagulant action in platelet poor plasma. One can however also reason that the primary use of a heparin is to catch thrombin, so that no platelets are activated. If this is a valid reasoning, then ACLM would be a better preventive drug than BCLM, whereas BCLM would be better in curative situations.

*Simple and composite effects of heparins*

The simple and direct effects of heparin in plasma are the catalysis of thrombin and factor Xa inhibition. The catalysis of thrombin inhibition is simply a function of the amount of circulating ACLM and its specific activity. Factor Xa inhibition is a function of the total amount of active HAM, i.e. of both the ACLM and the BCLM concentration. If we denote the specific activities by S, with a subscript indicating the relevant factor, then :

$$aIIa = S_2.[ACLM] \text{ and} \quad (a)$$

$$aXa = S_{10}.[ACLM] + S'_{10}.[BCLM] \quad (b)$$

Above we discussed how these formulas can be used to obtain the ACLM and BCLM concentrations if the specific activities are known.

Any effect of heparin on a biological variable is a consequence of its aIIa and/or aXa action. For the sake of simplicity we assume the aIXa action to be adequately rendered by the antithrombin action (see above). This means that e.g. the inhibition (prolongation) of the APTT (P(aptt)) is dependent upon both actions :

$$P(aptt) = p.\ aIIa + q.\ aXa \quad (c)$$

A combination of the above formulas (a, b and c) shows that :

$$P(aptt) = p'.ACLM + q'.BCLM \quad (d)$$

where p' and q' are combinations of p, q and the relevant specific activities. Because a pentasaccharide that has only aXa activity is a poor inhibitor of the APTT we can guess that p' is considerably greater than q' but further quantitation is impossible at the moment. This formula says simply that the outcome of a test like the APTT is dependent upon both the concentration of ACLM and of BCLM.

What holds for the APTT will hold for any other biological effect, but the coefficients (p' and q') will be different. If, e.g. we look at the inhibition of thrombus formation in a patient by heparin, it will again be a function of the ACLM and the BCLM concentrations, analogous to formula d. There is however no reason to assume that it will be precisely as dependent upon ACLM and BCLM concentrations as the APTT is. In other words, the coefficients (p' and q') in equation d will be different when this formula renders the APTT than when it is modified to render the antithrombotic action. The same will hold for the bleeding tendency caused by a heparin. Again the coefficients will differ from both those for the APTT and those for the antithrombotic activity.

It is even the bread and butter of heparin therapy that the coefficients for the bleeding tendency are smaller then those for antithrombotic action. This defines the therapeutic window. The clinically relevant coefficients will always be different from those governing the APTT or any other clotting test. Therefore any single *ex vivo* laboratory variable is incommensurable with antithrombotic or bleeding actions and it is fundamentally impossible to judge the effect of a heparin on basis of any single test only.

The approach that we propose is to determine ACLM and BCLM concentrations according to the principles exposed in previous paragraphs. Then we can relate antithrombotic and bleeding effects to those concentrations. In the meantime for a series of biological tests the dependence on ACLM and BCLM concentrations can be determined. That laboratory determination that reacts more or less in the same way as thrombosis inhibition or bleeding will then be the test of choice for routine monitoring of

patients. It may seem difficult to determine the influence of pure ACLM and BCLM on haemostasis and thrombosis, but this is not necessarily the case, because the high affinity fraction of unfractionated heparin is a good model for ACLM and the synthetic AT III binding pentasaccharide is a model for BCLM.

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