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Low molecular weight heparins – their mode of action in plasma and its consequences for the clinical laboratory

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Introduction

Unfractionated heparin (UFH) has been with us as a drug since the 1930s and low molecular weight heparins (LMWH) for some 20 years. This means that there is a fair amount of established practice in dealing with these drugs. Research in the last few years has made it clear that some of the traditional ways of approaching heparins are not necessarily the most adequate ones. Indeed there is a considerable amount of confusion arising from attempts to define LMWHs in terms that are adequate to deal with UFH. In this article we propose an approach to the clinical chemistry of heparins based on recent biochemical data. We will indicate what the differences are with the existing practice and we will show how existing practice can be brought in line with the new approach.

Types of heparin

Heparin is a mixture of linear polysaccharides consisting of alternating iduronic acid or glucoronate units and N-acetylglucoseamine units joined by glycosidic 1–4 linkages. Most of the N-acetyls of the glucosamine are replaced by N-sulfo groups. Most iduronates are O-sulfated in their 2-position, most glucosamines in their 6-position. The result is that there is a wide variety of different possible monosaccharides. They occur in almost random order and the chain lengths can vary from five to 100 or more. This chemical diversity is reflected in different biological activities.

In order to be active in antithrombin III (ATIII) dependent reactions (see below), heparin has to bind to ATIII via a specific pentasaccharide sequence. Heparins that contain this sequence we call high affinity material (HAM). The low affinity material (LAM) as such is not active in ATIII dependent reactions but may influence the action of HAM by replacing it in nonspecific reactions (see below). The HAM comes in two kinds: the material with a chain length of 5–17 monosaccharide units (MW 1700–5400) which is not capable of catalysing ATIII dependent thrombin activation. We therefore call it below critical chain length material (BCLM). The HAM longer

than 17 U, capable of catalysing thrombin inactivation, we called above critical chain length material (ACLM).

Biological activities of heparins

Apart from their anticoagulant action, heparins have a number of biological activities, such as inducing the release of lipoprotein lipase and tissue factor pathway inhibitor (TFPI) from the vessel wall. Here we will restrict ourselves to the effect of heparin on the blood coagulation process in plasma, because that is the basis for the assessment of the heparin effect in the clinical laboratory.

It is essential to distinguish between specific and composite biological properties of heparin, the former are the specific biochemical reactions in which heparin is active, the latter are consequences of this activity in composite reaction systems.

The specific biological property of a heparin is its catalytic action in the inactivation of clotting proteases by ATIII. There are three main candidates, thrombin, factor Xa and factor IXa. The inactivation of clotting factor XIa, XIIa and kallikrein is also accelerated by heparins but the effect is much smaller than that on the other factors. Also contact activation presumably is only of minor importance in haemostasis and thrombosis. We, therefore, will not discuss these factors further. Factor IXa inhibition, like thrombin requires ACLM, it contributes to the heparin effect in the intrinsic system. Factor IXa inhibition is not at the basis of, nor does it significantly contribute to, any current laboratory test so we will not discuss it either.

In practice we are left with two specific biochemical actions of heparin. The first is antifactor Xa activity, i.e., the property to catalyse the formation of an inactive complex between ATIII and factor Xa. The second is antithrombin activity, i.e., the property to catalyse the formation of an inactive complex between ATIII and thrombin. It is important to note that the first is a property of all HAM, whereas the second is a property of ACLM only. All heparins, and many other negatively charged polysugars catalyse the inactivation of thrombin by heparin cofactor II. For this such high concentrations of heparin are necessary as are rarely attained in clinical practice, except maybe in extracorporal circulation.

How to express heparin activity

Because the composite effects of heparin were known long before the specific effects, the standardisation has until now been carried out in terms of clotting tests. It was done by comparison in an arbitrary coagulation test to an arbitrary standard preparation. Now that the mode of action is known we can be more precise. HAM activity can be unequivocally quantitated by its antifactor Xa activity and in centimeter-gram-second compatible units. Analogously ACLM can be expressed by its antithrombin activity.

In plasma without heparin, factor Xa is inactivated by its reaction with ATIII. The reaction velocity is proportional to the concentration of ATIII and of factor Xa. Because in clotting plasma ATIII is present in μM quantities (concentration in normal plasma 2.24 ± 0.04 μM) and factor Xa in nM amounts (up to 20 nM), this means that factor Xa disappears with a half-life time ($t_{½}$) that is inversely proportional to the ATIII concentration. In other words, the decay constant, k_{dec}, is proportional to the ATIII concentration. Heparin increases k_{dec}, again proportionally to the HAM concentration. This is the basis for the definition of our standard and method independent unit of HAM activity. We define:

One unit of HAM activity is that amount of HAM that, in plasma with *n* μM ATIII increases k_{dec} of factor Xa by *n* min^{-1}.

Analogously we define:

One unit of ACLM activity is that amount of ACLM that, in plasma with *n* μM ATIII increases k_{dec} of thrombin by *n* min^{-1}.

It is important to note that the reaction conditions are defined to be as close as possible to the conditions in which heparin acts in vivo: normal plasma, diluted as few as the circumstances allow, and normal free Ca^{2+} concentration (1.15–1.32 mM).

Any LMWH preparation now can be assigned a specific activity. The specific activity is simply the amount of activity-units/unit weight (μG). Defining the specific activity allows the determination of heparin concentrations in plasma. If, after administration of HAM with a specific activity of S U/μG, we encounter a plasma sample in which we measure an HAM activity of Z U/ml than we can calculate the amount of HAM material as being Z/S μG/ml. Analogously we may calculate the ACLM content and then obtain the BCLM content as HAM minus ACLM.

Composite heparin effects

In the body or in isolated plasma or blood the specific heparin actions cause a variety of effects. In vitro, e.g., they prolong the whole blood clotting time, the contact activated clotting time, the activated partial thromboplastin time etc. In vivo they diminish a patients tendency to thrombosis and increase his tendency to bleed. All these are composite actions of heparin because they are influenced by both the antifactor Xa activity and the antithrombin activity. The relative contributions of both activities to the various composite effects are not exactly known as yet. There is no reason to assume that one composite action (e.g., the bleeding tendency), will depend upon the specific effects (antithrombin activity, antithrombin Xa activity) in the same manner as another (e.g., the antithrombotic effect). This is the rationale behind the search for heparins with a more favourable distance between therapeutically active- and potentially dangerous doses. Most tests use to screen for heparin activity in the

clinical laboratory (activated whole blood clotting time, APTT) respond to heparin in a composite way. Some current tests, such as the anti-Xa test and the thrombin time, are monospecific. Apart from technical difficulties they may serve to determine heparin activity in terms of new, standard independent units. They only have to be calibrated against standards defined in terms of new units.

Most heparins contain important amounts of material that does not bind to ATIII (LAM). This can influence ATIII dependent reactions because it replaces HAM in complexes with heparin binding proteins other than ATIII, such as histidin rich glycoprotein, vitronectin and heparin cofactor II. This ensures that adding LAM to plasma will free HAM from unproductive bindings and make it available for ATIII. Activated platelets shed platelet factor 4 (pf 4), that almost irreversibly binds heparins, especially those with a chain length of >17 U. Here again LAM may bind and save HAM for its ATIII-dependent actions.

Comparison of new and old units

Usually the potency of a given heparin is expressed by comparing it to that of a standard preparation in a given clotting test. The outcome of the clotting test is a composite effect of heparin. Different combinations of ACLM and BCLM may add up to the same final effect on such a test. A pure BCLM, like the synthetic ATIII-binding pentasaccharide, will, just like UFH prolong the APTT or any other clotting test (except the thrombin time) to any desired length when added in high enough quantities. Yet an amount of pentasaccharide that in a given test is equivalent to a given amount of UFH, will not, in vivo, have automatically the same antithrombotic effect as that amount of UFH. An equivalent effect of two doses of different heparins in one test, will not necessarily mean an equivalent effect in another test or in an in vivo situation. Therefore, the potency of a heparin, assessed by comparison via a clotting test, is ill defined. It depends both upon the nature of the standard and upon the way of comparing the activities. We calculated, e.g., that the activities given on the labels of LMWH vials, that are based on the current way of estimating antifactor Xa activity (in the absence of Ca^{2+} ions), overestimate the real heparin activity in plasma 1.37–1.76 times. The new units that we propose allow expression of heparin activity in a standard and method independent way.

The in vitro correlate to in vivo heparin action

The essential properties of a heparin, i.e., its in vivo antithrombotic and antihaemostatic properties are also composite actions to which the different specific actions contribute. Often it is assumed, and almost always it is tacitly implied, that a test like the APTT will react to a heparin in the same way as the in vivo antithrombotic and antihaemostatic action. This is false, as can be easily demonstrated from the fact that, e.g., dermatan sulfate is a good antithrombotic that hardly prolongs the APTT.

The search for a good laboratory correlate to the in vivo actions of heparin has occupied us for several years. We have good reasons to believe that the surface under the thrombin generation curve (the endogenous thrombin potential, ETP) may serve as such. Because thrombin is an enzyme, the amount of "work" that it can do is proportional to its concentration and to the time that it is active. The ETP increases and decreases proportionally with the amount of thrombin generated in clotting blood, as well as with the time that it remains active. Indeed the ETP, unlike the APTT and other clotting times, is decreased by all anticoagulant therapies (oral anticoagulation, heparins, hirudin, dermatan sulfate) and is increased in congenital prothrombotic conditions like ATIII deficiency.

We recently developed an easy way to determine the ETP in a single spectrophotometric test. In this test an inefficient chromogenic substrate for thrombin is added to the plasma, before coagulation is triggered. The thrombin that generates and disappears will continuously convert some of the substrate, but never more than about 10% of the total amount added. In this way the final amount of chromogenic substrate converted is proportional to the concentration-time integral of thrombin.

Heparin pharmacokinetics and pharmacodynamics

The effect of heparin administration to a patient until now has usually been determined by clotting tests, i.e., the effect of heparin on the blood coagulation mechanism was tested rather than the amount of heparin present. With the approach sketched above it is possible to determine first the levels of ACLM and BCLM after heparin administration and then register their effects on composite phenomena such as clotting times, thrombin formation, thrombus growth and bleeding tendency. In this way it is therefore possible to separate heparin pharmacokinetics from its pharmacodynamics.

The relative importance of anti-Xa and antithrombin activity

Compared to UFH, LMWHs are enriched in BCLM. This means that their antifactor Xa activity will be relatively more important than their antithrombin activity. Especially when expressed by comparison to a heparin standard this effect seems important. By definition one unit of standard heparin contains one unit of antifactor Xa activity and one unit of antithrombin activity. This tends to obscure the fact that the antifactor Xa activity in plasma (normally 0.49 ± 0.02 $min^{-1}/\mu M$ ATIII), both in the presence and in the absence of ACLM is always some three times lower than the antithrombin activity (normally 1.508 ± 0.02 $min^{-1}/\mu M$ ATIII). The only way to enhance the antifactor Xa activity in plasma relative to its antithrombin activity is by adding BCLM. That is what happens when LMWHs are administered. But is enhancing the antifactor Xa activity in plasma an efficient means of inhibiting thrombin production, admittedly the only possible effect of enhancing antifactor Xa

activity? The answer is, that it is not. The reason for this is easy to understand. The prothrombin converting enzyme consists of a stoichiometric complex of factor Xa and factor Va adsorbed onto procoagulant phospholipids. The factor present in the lowest concentration limits the prothrombinase activity. This is factor Va. Actually factor Xa is present in clotting plasma in an about 10-fold excess. This means that one can inhibit some 90% of the factor Xa activity without altering the velocity of prothrombin activation in clotting plasma. Factor Xa inhibition therefore is an inefficient means of inhibiting thrombin production. This does not mean to say that it will not work. Pure BCLMs, like the synthetic pentasaccharide, can be given in such high amounts as to be efficient anticoagulants and hence efficient antithrombotics. It does mean however that the relative enrichment of BCLM over ACLM in LMWHs has not much influence in vitro. Nevertheless, in vivo there are two circumstances which favour the effect of the BCLM injected with LMWHs. In the first place the biological half-life time of BCLMs is significantly longer than that of ACLMs, so that a "fractionation in vivo" takes place, this means that the relative amount of BCLM circulating increases with time after injection. The second is that activated platelets release platelet factor 4, that very efficiently neutralises ACLM but not BCLM. Haemostasis and thrombosis inevitably entails platelet activation, hence pf 4 release. In this way BCLM, but not ACLM, may escape neutralisation at precisely those sites where thrombosis occurs.

How to monitor heparin therapy

If we need information on the heparin effect in a clinical situation we need one or more of three things: 1) a quantitative estimate of the overall effect in this patient at this moment; 2) the level of ACLM; and 3) the level of BCLM. Neither of these are presently available. Often the APTT is used to assess the overall effect. It has the advantage of being easy to determine and familiar. It is relatively insensitive to BCLMs and varies greatly with a large number of conditions in the patients plasma. It also is not very precise. For assessing HAM (ACLM + BCLM) levels the antifactor Xa activity tests are in common use. They are useful tests but it should be taken into account that the results are obtained in the absence of Ca^{2+} ions. This means that the absolute values are overestimated when expressed in terms of standard UFH or any standard that in its turn is compared to standard UFH. Proper standardisation of the method could avoid these problems. Often the thrombin time (TT) is used as an antithrombin activity test. It is relatively specific but imprecise. There is great need for a good antithrombin test for clinical use, capable of reliably measuring the ACLM content.

The current opinions on the necessity and value of laboratory control are obtained with deficient tests and therefore cannot be claimed to be optimally founded. Yet one may summarise the situation as follows:

1. In prophylactic heparin administration one can resort to standard dosage and control of the heparin effect is not necessary; at least it is not useful with the

available tests because no correlation has been found with bleeding or (re)thrombosis.

2. There are indications that adjustment of therapeutic heparin doses to body weight ameliorates the results of the therapy. If adjusted doses are better than standard doses, then it might be expected that adjustment on the effect of the administration is better than adjustment on body weight. At present control of the APTT or of antifactor Xa activity levels are used to control therapeutic heparin treatment. The correlation with bleeding and/rethrombosis is poor.
3. In extracorporeal circulation there is a great need for specific heparin tests, especially because often drugs are given (plasma expanders, aprotinin etc.) that interfere with coagulation tests.

References

This type of publication does not allow extensive reference to the original literature. Recently a book was published that covers the larger part of the relevant material:

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Further information is to be found in:

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