Depletion of Lipoprotein Lipase After Heparin Administration

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Some or most of the turnover of lipoprotein lipase (LPL) occurs by dissociation from vascular endothelial sites in extrahepatic tissues and further degradation in the liver. Heparin greatly enhances this dissociation and delays but does not abolish uptake in the liver, raising the possibility that heparin could lead to accelerated catabolism of functional LPL. To investigate this, we determined time curves for heparin (anti-factor Xa activity) and for LPL and hepatic lipase after injection in rats of two doses of conventional unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH). The high dose (250 U/kg) of both heparins resulted in similar initial levels of LPL activity in plasma, but at 30 minutes the activity with LMWH had declined by more than 80%, whereas with UFH it remained essentially unchanged during this time. In contrast, time curves for heparin activity in blood were similar for the two heparins. The low dose (50 U/kg) led to lower initial levels of LPL activity with LMWH in spite of slower elimination of heparin activity from the blood. These results agree with previous studies that indicate that LMWH has a similar ability as UFH to release LPL, but a lesser ability to delay its removal by the liver. Only slight differences were noted in the time curves for hepatic lipase with the two heparins. To assess the possible depletion of the lipases, we administered a second large dose of conventional heparin. One hour after the first injection, the second injection resulted in lower plasma LPL activities in all four groups. This depletion of releasable LPL was more pronounced with high-dose LMWH (49% of that in saline-treated controls versus about 60% in the other groups). The LPL activity released by the second injection remained significantly depressed with low-dose LMWH and the high dose of either heparin at 4 hours, but had returned to normal after 24 hours. By contrast, no depletion of hepatic lipase activity could be shown at any time. The results showed that release of LPL into the circulating blood is followed by a period during which time the stores of functional LPL are depleted. This occurs with both UFH and LMWH; the difference between the two heparins lies more in the kinetics of the LPL removal process than in its ultimate result. (Arterioscler Thromb. 1993;13:1391-1396.)

Key Words • low-molecular-weight heparin • hepatic lipase • endothelium • lipase clearance • heparin clearance • in vivo • rats

Heparin has been used clinically for about 50 years, and its efficacy in the prophylaxis and treatment of thrombosis is well documented. In addition to its anticoagulant activity, heparin greatly increases the activity of two lipases in plasma: lipoprotein lipase (LPL) of extrahepatic origin, which hydrolyzes mainly triglycerides in chylomicra and very-low-density lipoproteins, and hepatic lipase (HL), which acts preferentially on remnants from chylomicra and very-low-density lipoproteins and on high-density lipoproteins. The "lipolytic effect" of heparin comes from its ability to displace the two lipases from their endothelial ligand, heparan sulfate, to form heparin-lipase complexes in the plasma. The interaction of heparin with the lipases is thought to be mediated by the interaction of negative charges on heparin with positively charged amino acid residues in the enzymes.

Low-molecular-weight heparins (LMWHs) have attracted attention because of their similar antithrombotic activity but lesser tendency to cause bleeding compared with unfractionated heparin (UFH). LMWH has been found to cause lower lipase activities in plasma than does UFH, but LMWH is also reported to cause higher lipase activities. Liu et al recently reported that the lower LPL activity could be attributed mainly to a lesser ability of LMWH to prevent hepatic clearance of the lipase, whereas the release of LPL from extrahepatic tissues was similar with both heparins. An implication is that rapid clearance of LPL after LMWH could lead to depletion of available LPL. Two recent reports support this hypothesis. Even a transient depletion could impair the catabolism of triglyceride-rich lipoproteins.

To investigate these relationships we determined time curves for heparin and for the two lipases after injection in rats of LMWH or UFH at two clinically relevant doses. To assess the possible depletion of lipases after the heparin injections, we administered a second large dose of heparin to determine the amount of the lipases remaining at endothelial sites. The results showed a temporary depletion for LPL but not for HL. The depletion occurs with both UFH and LMWH; the
difference between the two heparins lies more in the kinetics of the process than in its ultimate result.

Methods

Materials

The heparins used in this study were from Novo Nordisk A/S, Bagsvaerd, Denmark. The trade name for the LMWH is Logiparin. It is produced by enzymatic depolymerization of pig mucosal sodium heparin using heparinase from Flavobacterium heparinum. The doses of LMWH were the same as in a previous study,\textsuperscript{10} 50 and 250 U/kg body wt, which correspond, respectively, to approximately 0.65 and 3.25 mg/kg body wt. The doses of UFH were chosen to give the same anticoagulant activity, ie, 50 and 250 U/kg body wt, which correspond, respectively, to 0.30 and 1.50 mg/kg body wt. Injection solutions were prepared by chromatography on protein A-Sepharose from heparin perfusates of rat liver, partially purified by heparin-Sepharose chromatography. Immunoglobulins were prepared by chromatography on protein A-Sepharose (LKB-Pharmacia Biotechnology, Stockholm, Sweden) and a solution of about 5 mg/mL made up in 10 mmol/L tris(hydroxymethyl)aminomethane (Tris)-HCl and 0.15 mol/L NaCl, pH 7.4.

Animal Procedures

Male Sprague-Dawley rats (Moellegard Breeding Center, Denmark) weighing 180 to 220 g were used. The animals were housed with free access to standard pellets and water in a 12-hour light cycle. Food was removed the evening before the start of the experiment. In the group in which effects of heparins were assessed after 24 hours, the animals were allowed to eat the night before the experiment and fasted after the injection of the heparin preparations. This procedure was chosen to avoid a too-long duration of the fast, which might in itself modify lipase activities. All animals were injected between 8 and 12 AM. The test preparations (1 mL/kg body wt) were injected in a tail vein to unanesthetized rats, except for rats in the 5- and 10-minute groups. These animals were anesthetized before injection of the test substance. Ten minutes before the designated times the animals were anesthetized by intramuscular injection of Hypnorm Vet (AB Leo, Helsingborg, Sweden; 0.5 mL/kg body wt) and diazepam (2.5 mg/kg body wt). Hypnorm Vet is a combination of 0.315 mg of the narcotic analgesic fentanyl citrate and 10 mg of the tranquilizer fluanisone in 1 mL of solution. Blood samples were taken from the left jugular vein. One aliquot (−0.2 mL) to be used for lipase assay was transferred to a heparinized tube (final concentration, 200 U heparin/1 mL blood). Another aliquot, to be used for assay of heparin activity, was transferred to a tube containing trisodium citrate (final concentration, 13 mmol/L). All animals procedures were approved by the Animal Ethics Committee of the University of Umeå.

Assays

Detailed protocols for the lipase assays have been published.\textsuperscript{14} Plasma was separated by centrifugation at 4°C. For assay of LPL the plasma was first incubated on ice with anti-HL immunoglobulin G in a ratio of 1:1 (vol/vol) for 2 hours. The substrate emulsion was Intraplast 20% (Kabi-Pharmacia Hospital Care, Stockholm, Sweden), into which a trace amount of tri-9,10-[\textsuperscript{3}H]-oleoylglycerol had been incorporated by sonication. On the day of the determination, incubation medium was prepared from 10 μL emulsion, 10 μL heat-inactivated rat serum as a source of apolipoprotein C-II, and 100 μL of a mixture containing 12% bovine serum albumin (fraction V, Sigma, St Louis, Mo), 30 U standard heparin, and 0.3 mol/L Tris-HCl, pH 8.5. The sample volume was 5 μL. The total volume was adjusted to 200 μL with distilled water. Incubation was for 30 minutes at 25°C in a water bath. The activity of HL was determined by using a gum arabic-stabilized emulsion of labeled triolein in purified unlabelled olive oil. The assay medium contained 1 mol/L NaCl to suppress LPL activity, and no source of apolipoprotein C-II was added. Plasma samples (5 μL) and 150 μL substrate emulsion were adjusted to 200 μL with distilled water and incubated at 25°C for 30 minutes. All incubations were performed in triplicate. The reactions were stopped, and the fatty acids were extracted and counted in an LKB Rack beta counter. One milliunit (mU) of lipase represents 1 nanomole of fatty acids released per minute.

Heparin activity was determined on citrated plasma by a modification of the technique originally described by Teien et al.\textsuperscript{15} The method, which was automated on a Corona Analyser (Clinicon AB, Sweden), measures the ability of heparin to potentiate the inhibition of factor Xa (bovine; Chromogenics, Göteborg, Sweden) by antithrombin III (human; Chromogenics) using a chromogenic substrate (S2222, Chromogenics).

Statistics

The significance of differences between experimental groups was evaluated after an analysis of variance (ANOVA) by Newman-Keuls test (P<.05) using the NCSS program (Kaysville, Utah). Data are presented as mean±SEM; in most cases n=6 rats.

Results

Clearance of Heparin Preparations

Heparin clearance was studied in the same rats as lipase activities (Fig 1). Heparin was assayed by factor Xa inhibition on the assumption that this paralleled the decline in chemical concentration. Injection of the high dose (250 U/kg) resulted in similar initial anti–factor Xa activities for both LMWH and UFH. The activities then declined rapidly to reach close to basal values by 2 hours with no significant differences between the two preparations. After injection of the lower dose (50 U/kg), the initial anti–factor Xa activity was higher for LMWH than for UFH (significant at 5 minutes). From 30 minutes there were no significant differences between the two heparins, and by 1 hour the anti–factor Xa activity had returned to control levels. To compare the clearance of high and low doses, we plotted the data on a log/linear graph that shows that clearance of both heparins followed first-order kinetics. Calculated fractional catabolic rates for the high doses were 1.74 pools/h\textsuperscript{-1} and 1.40 pools/h\textsuperscript{-1} and for the low doses 2.40 pools/h\textsuperscript{-1} and 1.74 pools/h\textsuperscript{-1} for UFH and LMWH,
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Time (hours)

Fig 1. Top: Graph showing elimination curves for heparin preparations. Low-molecular-weight heparin (LMWH; ---) and unfractionated heparin (UFH; ——) were given intravenously to rats at two doses: LMWH 50 U/kg (▲) and 250 U/kg (●); and UFH 50 U/kg (○) and 250 U/kg (■). Blood was taken at the indicated times and assayed for heparin activity by an anti-factor Xa (anti Xa) assay (U/mL). Data are mean ± SEM of six rats. For the high dose there were no significant differences between the two heparins at any time. For the low dose the values were significantly higher for LMWH at 5 minutes, but not thereafter. Bottom: Same data were plotted on a semilogarithmic scale to illustrate the first-order kinetic of the heparin clearance.

respectively. The fractional catabolic rates were higher for the low doses, which suggests that removal may be a saturable process. The present data, however, are insufficient to define the disappearance accurately.

Release of Lipase Activities

Fig 2 shows time curves for plasma lipase activities after the two heparins. In the time curves for HL the only significant difference between the heparins was a lower activity at 30 minutes after the high dose of LMWH compared with UFH (Fig 2). This was matched by a lower heparin concentration, so that after the high dose of either heparin, HL and heparin activities followed each other closely. The HL activities had returned to basal levels 1 hour after the low dose and 2 hours after the high dose of either heparin. In all, there were only minor differences between the two heparins in relation to HL activity.

Five minutes after the high dose (250 U/kg) of LMWH or UFH, the levels of LPL activity in plasma were similar (Fig 2). The activity then remained essentially unchanged until 30 minutes with UFH, whereas with LMWH it declined by more than 80% during this time. After 1 hour, the LPL activity had almost returned to basal with LMWH (21 ± 3 mU/mL compared with 11 ± 2 mU/mL), whereas it remained elevated with UFH (81 ± 26 mU/mL). The low doses of the heparins (50 U/kg) resulted in lower LPL activities; at 5 minutes, LPL activity was significantly lower with LMWH (only two thirds of the activity obtained with UFH). With either heparin, LPL activities then decreased rapidly during the following 30 minutes and were close to basal after 1 hour.

During the first hour, when heparin and lipase activities were substantial, at a given heparin concentration LMWH was always associated with lower LPL activity than UFH both after the high or the low dose. Furthermore, there was a marked discrepancy between the LPL activities at the same heparin concentration, which was reached soon after injection of the low dose or late after
Fig 3. Bar graphs showing lipoprotein lipase (LPL; top) and hepatic lipase (HL; bottom) activities after administration of standard heparin in rats previously injected with low-molecular-weight heparin (LMWH) or unfractionated heparin (UFH). Same rats as in Fig 1. Ten minutes, 1 hour, 4 hours, and 24 hours after injection of LMWH or UFH at two doses (LMWH 50 U/kg, LMW50; LMWH 250 U/kg, LMW250; UFH 50 U/kg, UFH50; UFH 250 U/kg, UFH250), the rats were injected with standard heparin (835 U/kg). Solid bars represent the lipase activities in plasma before the second injection. Hatched bars represent the increase in lipolytic activities after the second injection. Hence, the total height of the bars represents total plasma lipase activities. Data are the mean±SEM of six rats for each group.

injection of the high dose. For instance, similar heparin concentrations, around 1 anti–factor Xa U/mL, were reached 5 minutes after injection of the low-dose LMWH and 1 hour after injection of the high-dose LMWH (Fig 1). The LPL activity was three times higher in the first case than in the second. A similar, but less marked, tendency was seen for HL, ie, the same heparin concentration gave higher HL activities soon after injection of the low dose but later after injection of the high dose.

Depletion of Lipase Activities After Heparin

We first administered LMWH or UFH as above, and then assessed the remaining amount of heparin-releasable lipase by injecting a large dose of standard heparin (835 U/kg). This dose released significantly more lipase than the 250 U/kg dose of either LMWH or UFH. For instance, compare LPL activities in saline-treated controls in Fig 3 (about 450 mU/mL) to data for the 250 U/kg dose in Fig 2 (about 350 mU/mL). The amount of the lipases released by the large dose of standard heparin changed somewhat after saline injection (Fig 3). The released LPL activities (upper panel) increased significantly from 10 minutes to 4 hours and then decreased at 24 hours. The HL activities (lower panel) did not change from 10 minutes to 4 hours, but they decreased slightly at 24 hours.

Ten minutes after administration of LMWH or UFH, the injection of the high dose of standard heparin resulted in similar plasma LPL activities for the four preparations that were close to the activity released in saline-treated controls (Fig 3). There was a tendency toward lower activity in the low-dose LMWH group, but this did not reach statistical significance. After 1 hour, the second injection of heparin (835 U/kg) resulted in lower plasma LPL activities in all four groups that were 60% or less of those in saline-treated controls. The high-dose LMWH group showed significantly lower plasma LPL activity than the other three groups (49% compared with about 60% of controls). The rats given the high doses of LMWH or UFH still had elevated LPL activities in plasma. The net increase caused by the second heparin injection did not differ between these groups (45% and 50% of the increase in saline-treated rats), but was significantly less than in the rats given the low dose of either heparin (60% of control value). Four hours after the first injection, the large heparin dose resulted in significantly lower plasma LPL activities in the high-dose LMWH group (75% of controls) and in both the UFH groups (82% for the low dose and 80% for the high dose). After 24 hours there were no significant differences in the LPL activities released by the second injection of heparin.

The effects of the heparin preparations on HL differed from the effects on LPL (Fig 3). Ten minutes after
the first injection, the sum of circulating and releasable HL activities was similar to control for all four groups. For LPL a marked depletion was noted in all four groups 1 hour after the first injection and was most marked for the high-dose LMW group. For HL a significant depletion was seen only for the net increase in the high-dose group and was only about 15% compared with about 40% for LPL. There were no significant differences in HL activities released by the second heparin injection at 4 or 24 hours.

Discussion
This study showed that administration of a single dose of heparin in a clinically relevant range leads to temporary depletion of available HL. Hence, there are two aspects to the effects of heparin on this lipase. Heparin first releases the lipase into blood, where it can more efficiently act on the plasma lipoproteins. This is the well-established “clearing reaction.” The new finding is that when the released lipase has disappeared from the blood there follows a period during which lipase stores are functionally depleted.

LPL activity disappeared more rapidly from blood than than heparin activity did. This was true for both heparins, but was more pronounced with LMWH. For instance, from 5 to 30 minutes after injection of high-dose LMWH, LPL activity decreased by more than 85%, whereas hepatic activity decreased by only 65%. Presumably LPL, after release into the blood by heparin, is likely to be taken up by and degraded in the liver. Because of this, there was no longer enough lipase in the peripheral tissues at 30 minutes to support blood LPL activities in proportion to the hepatic levels.

In chronically heparinized rats, Goldberg and Chajek-Shaul described increased plasma lipolytic activity together with depletion of endothelium-bound LPL. They attributed the increased serum LPL activity to continuous displacement of newly synthesized LPL to the circulation rather than to a delayed clearance of the enzyme by heparin. Similarly, Rassin et al. have recently reported that in patients receiving low-dose heparin infusions, the amount of LPL that can be released by a large bolus of heparin is decreased. One would need more time points than we have to accurately define the duration of the depletion. In rats given low-dose LMWH, heparin-releasable LPL returned to control levels at 4 hours. In the other three groups the activity had increased but was still below control at this time. Studies on turnover of tissue LPL activities generally indicate that the enzyme is turned over quickly; hence, one expects relatively rapid recovery of LPL activity after heparin has disappeared from the blood.

In contrast to results with LPL, injection of heparin did not appear to significantly modify the available HL activity. The sum of the activity in the blood and the activity that could be released by a second injection of heparin was not changed much. This suggests that HL that had been displaced into the circulation returned to its binding sites when heparin disappeared from the blood. HL activity in preheparin plasma has been found to correlate closely to postheparin HL activity, which would be the case if there were an equilibrium between HL circulating in the blood and HL at binding sites in contact with blood. Hence, it appears that the effect of heparin on HL is merely to shift the equilibrium distribution toward the blood, whereas for LPL, heparin enhances catabolism of the enzyme. HL is turned over more slowly than LPL; Schoonderwoerd et al. estimate a half-life of 4.6 hours. An implication is that the sites where HL and LPL bind in the liver are, at least functionally, different. For HL, the binding sites probably do not mediate rapid degradation but serve mainly to hold the lipase in place for interaction with lipoproteins. In contrast, binding of LPL in the liver is rapidly followed by internalization and degradation.

A previous study by Liu et al. indicates that LMWH is less effective than UFH in preventing hepatic uptake of LPL. This implies that depletion of LPL activity might be more pronounced after LMWH. That this factor came into play in the present experiments is shown by the more rapid decrease of plasma LPL activity after high-dose LMWH than after UFH. This presumably reflects more rapid hepatic clearance. However, there was no corresponding difference in depletion of LPL, as evaluated in the experiments with a second injection of heparin. The most likely interpretation of this is that once LPL is released into the blood by heparin, the enzyme will ultimately be taken up by the liver. If so, the difference between LMWH and UFH lies in the kinetics of the process, not in its ultimate result. It should be pointed out that the doses of LMWH and UFH were chosen on the basis of anti-factor Xa activity, reflecting clinical practice. On a mass basis more than twice as much and on a molar basis more than six times as much LMWH was given. The effects on the more easily interpreted HL activities were similar for these doses of LMWH compared with UFH. For LPL the initial release was also similar. The lipase activities evoked by different LMWH preparations differ considerably. The dose relations presumably depend on the detailed composition of the heparin preparations.

A clinically important consideration is the effect that temporary depletion of LPL activity has on lipoprotein metabolism. In a previous study Liu et al. found decreased clearance of a fat emulsion 1 hour after administration of an LMWH preparation, whereas clearance was enhanced at the same time after UFH. In that study plasma LPL activity had returned to basal values in rats given LMWH, so that they were, presumably, in the lipase depletion phase. The rats given UFH, on the other hand, still had high levels of circulating LPL, thus explaining the rapid clearance of the fat emulsion. Had the experiments been repeated at 2 hours one might have found a decreased clearance in rats given UFH. Liu et al. used a narrow-sized fraction of heparin fragments that corresponded mainly to de-casacharides. In contrast, the present study used a commercial LMWH preparation. This preparation is rather polydisperse, which has pharmacokinetic advantages. There is an overlap of around 25% between the molecule size range in the two heparin preparations. It is likely that this overlap blunted the differences between the two. Hence, caution should be exercised in extrapolating the present results to other LMWH preparations. Direct experiments should be performed to evaluate the effects on lipoprotein catabolism.

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