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Low-M_r heparin is as potent as conventional heparin in releasing lipoprotein lipase, but is less effective in preventing hepatic clearance of the enzyme

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This study compares a low-M_r heparin preparation with conventional heparin with respect to its interaction with lipoprotein lipase (LPL) *in vitro* and its effects on the enzyme *in vivo*. Both heparin preparations were polydisperse in binding to LPL, but on average the low-M_r preparation showed lower affinity. Thus both conventional and low-M_r heparin bound quantitatively to immobilized LPL, and were eluted as broad peaks when a salt gradient was applied, but the peak for low-M_r heparin was shifted towards lower salt concentrations. To displace LPL from immobilized heparin a higher concentration of low-M_r than of conventional heparin was needed. Injection of the low-M_r heparin into intact rats resulted in lower plasma LPL activity than did injection of an equal mass of conventional heparin, but when the liver was excluded from the circulation both heparin preparations resulted in similar plasma LPL activities. In perfused rat hearts, low-M_r heparin had at least the same effect on the release of LPL activity as did conventional heparin. In perfused livers, on the other hand, low-M_r heparin was less effective than conventional heparin in preventing the rapid uptake of exogenous labelled LPL. Hence the apparently lower average affinity of low-M_r heparin for LPL does not result in a demonstrably lower potency to release the enzyme from endothelial binding sites in peripheral tissues, but does result in a substantially decreased effect on the hepatic clearance of the enzyme.

INTRODUCTION

In addition to its anticoagulant activity, heparin also has a 'lipolytic' activity, which is exerted through release of lipoprotein lipase (LPL) and hepatic lipase from the vascular endothelium (reviewed by Olivecrona & Bengtsson-Olivecrona, 1989). This release results in rapid hydrolysis of lipoprotein triacylglycerols in the circulating blood, with generation of high levels of non-esterified fatty acids (Persson *et al.*, 1985) and conversion of the lipoproteins into remnant particles. Preparations of low-M_r heparin have attracted attention because they have improved pharmacokinetic properties (higher bioavailability and longer half-life) than conventional heparin (Bratt *et al.*, 1986; Mätzsch *et al.*, 1987; Holmer, 1989) and because they have similar anti-thrombotic activity as conventional heparin but in some animal models a lower tendency to cause bleeding (Carter & Kelton, 1982; Abdriuoli *et al.*, 1985; Holmer, 1989). They have also been reported to possess lower lipolytic activity (Kakkar *et al.*, 1982; Etienne *et al.*, 1983; de Swart *et al.*, 1984; Persson *et al.*, 1985, 1987), which in turn results in lower plasma levels of non-esterified fatty acids (Persson *et al.*, 1985). The molecular mechanisms behind the lower plasma LPL activity caused by low-M_r heparin are not well understood. One obvious possibility is that low-M_r heparin has a lesser ability to release the lipases (de Swart *et al.*, 1984), but this has been questioned (Harenberg *et al.*, 1985).

LPL is normally cleared from the circulating blood by the liver (Wallinder *et al.*, 1979, 1984; Vilaró *et al.*, 1988; Chajek-Shaul *et al.*, 1988*a*). This is a very efficient process; in the rat more than 50% of perfused LPL is removed by the liver in a single pass (Vilaró *et al.*, 1988; Chajek-Shaul *et al.*, 1988*a*). This hepatic removal is slowed down, but not abolished, by heparin (Vilaró *et al.*, 1988). Thus the reason for the high LPL activity in plasma after injection of heparin is 2-fold; heparin displaces the lipase from endothelial binding sites in peripheral tissues, and also slows down its removal by the liver. In the present study, we have examined the effect of low-M_r heparin on these two aspects of LPL turnover separately, in perfused hearts and livers respectively. The results show that the main difference between low-M_r and conventional heparin is in the ability to slow down removal of LPL by the liver, not in the ability to release the enzyme from peripheral tissues.

MATERIALS AND METHODS

Materials

Low-M_r heparin (LHN-1; Logiparin) was produced by Novo Nordisk A/S, Bagsvaerd, Denmark, by enzymic depolymerization of pig mucosal sodium heparin using heparinase from *Flavobacterium heparinum*. This preparation, lot F537II, had a peak maximum M_r of 4400, an anti-(factor Xa) activity of 91.8 units/mg and an anti-(factor IIa) activity of 50.3 units/mg. The M_r was determined by gel-permeation chromatography using heparin fractions as calibration standards, and the biological activities were determined by chromogenic substrate assays using the 1st International Standard for low-M_r heparin (National Institutes for Biological Standards and Control, London, U.K.) as reference standard. Conventional heparin (prepared from pig mucosa) was purchased from AB Lövens, Malmö, Sweden. Stock solutions containing 10 mg of heparin/ml were made in phosphate-buffered saline (20 mM-sodium phosphate/0.15 M-NaCl, pH 7.4). Various concentrations were obtained by appropriate dilution before use. Comparison of the two heparins was made on basis of mass. LPL was purified from bovine milk (Bengtsson & Olivecrona, 1977), radiolabelled with ^{125}I and repurified as previously described (Wallinder *et al.*, 1984). The preparations used in this study had specific radioactivities of about 20000 c.p.m./ng of LPL protein. LPL–

Abbreviation used: LPL, lipoprotein lipase.
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agarose was made by coupling LPL to cyanogen bromide-activated Sepharose (Pharmacia, Uppsala, Sweden). For this, LPL was first dialysed against 0.1 M-$NaHCO_3$/1.5 M-NaCl, pH 8.3, for 6 h at 4 °C. Then, 9.5 mg of dialysed LPL in 23 ml of buffer was mixed with 6 ml of wet activated gel. More than 90% of the added protein had bound after 18 h at 4 °C, as judged from the A_{280} of the liquid phase. Remaining activated groups on the gel were blocked by 5% ethanolamine in 0.1 M-$NaHCO_3$/0.5 M-NaCl, pH 9 (4 h, 4 °C). The gel was washed with coupling buffer, then with 10 mM-Bistris, pH 6.5, containing 1.5 M-NaCl and finally with Bistris buffer containing 0.15 M-NaCl, 0.02% (w/v) NaN_3 and 1 mg of conventional heparin/ml. The gel could be stored in this buffer for 2 weeks without much loss in binding affinity. Experiments aimed at comparing the binding affinities of the two different heparin preparations for the immobilized LPL were performed in parallel on the same day. Heparin–agarose was prepared as previously described (Bengtsson & Olivecrona, 1977). The heparin used was an unbleached crude pig mucosal heparin (Diosynth, Oss, Holland), which was purified as described (Lindahl *et al.*, 1965). Heparin was determined spectrophotometrically by the dimethyl Methylene Blue binding assay described by Farndale *et al.* (1982). For samples from column fractions with elevated concentrations of NaCl the effect of salt was compensated for by use of standard curves at different salt concentrations. Antiserum to rat hepatic lipase was raised in rabbits (Peterson *et al.*, 1985). An IgG fraction was used in this study.

Animal procedures

Male Sprague–Dawley rats weighing 250–430 g were supplied by A-lab, Stockholm, Sweden. The rats were housed in groups of five with access to standard pellet diet and water *ad libitum* in a 12 h light cycle. The animals were anaesthetized with Hypnorm Vet (AB Leo, Helsingborg, Sweden; 1 mg/kg body wt.), except those used for heart perfusion. These rats were lightly anaesthetized with diethyl ether. All experimental procedures were approved by the local Animal Ethics Committee.

For studies *in vivo* jugular veins on both sides were exposed in anaesthetized animals and heparin in 200 μl of 0.9% NaCl was injected rapidly. Post-heparin blood samples (200 μl) were withdrawn at the indicated times. Plasma was separated by centrifugation at 4 °C and incubated with anti-(hepatic lipase) IgG in a ratio of 2:1 (v/v) on ice for 2 h.

Supradiaphragmatic rats were prepared according to Bezman-Tarcher & Robinson (1965), but without dividing the rats into lower and upper parts.

For heart perfusion studies the chest of an anaesthetized rat was opened and the aorta was cannulated in ice-cold 0.9% NaCl. The heart was then perfused retrogradely at 37 °C as described by Chajek-Shaul *et al.* (1988*b*) with the use of an artificial 'lung' as described by Hamilton *et al.* (1974). The heart was first washed free of blood with a single pass of 20 ml of medium B (Eagle's minimum essential medium supplemented with 3% BSA, 1 mg of glucose/ml and 1 mM-glutamine). Then the heart was perfused with 25 ml of the same medium in recirculating mode for 10 min at 37 °C. The heartbeat resumed immediately when warm medium was passed through and remained stable at 198 ± 6 beats/min throughout the experiment. Perfusion pressure was maintained at 60 mmHg (8 kPa) by adjusting the pump speed. Portions of the perfusate were taken at the indicated times for determination of LPL activity. It was found that the loss of LPL activity was less than 20% when medium was incubated at 37 °C for 2 h if heparin was present at 0.2 μg/ml.

Livers were perfused as described by Hamilton *et al.* (1974), with some modifications. Perfusion was started immediately after cannulation of the portal vein with a flow rate of 2–3 ml/min. By making a cut in the inferior vena cava below the kidneys the flow rate was increased to about 25 ml/min to deliver sufficient oxygen to the liver. The medium was Krebs–Hensleit bicarbonate buffer, pH 7.4, containing 0.5% BSA and 1 mg of glucose/ml at 37 °C (medium A). The medium was passed through an artificial lung (Hamilton *et al.*, 1974) for oxygenation. After cannulation of the hepatic vein through the right atrium, the inferior vena cava above the right kidney was tightly ligated and the liver was excised. About 70 ml of medium A was used to flush the liver free of residual blood and the liver was then put into the perfusion chamber and perfused in recirculation mode with medium B. The total volume was about 25 ml. The medium was recirculated for 10 min before heparin was added. Labelled bovine LPL was added 2 min later. Samples were taken at the indicated times for determination of trichloroacetic-acid-precipitable radioactivity (Wallinder *et al.*, 1984). It should be noted that the flow rate (25–30 ml/min per liver) chosen in the present study frequently resulted in high portal pressure, over 30 cmH_2O, and that a certain swelling of the liver took place.

Assay of LPL activity

Conditions for the assay are described elsewhere (Peterson *et al.*, 1985). Briefly, the incubation medium was prepared on the day of determination by mixing 10 μl of substrate emulsion {tri-[9,10-^{3}H]oleoylglycerol sonicated into 20% Intralipid (Kabi Nutrition, Stockholm, Sweden)} with 10 μl of heat-inactivated rat serum and 100 μl of mixture containing 12% BSA (fraction V; Sigma, St. Louis, MO, U.S.A.), 30 units of conventional heparin and 0.3 M-Tris/HCl, pH 8.5. The total volume was adjusted to 200 μl with water, and incubation was carried out for 30 min at 25 °C in a water bath. The reaction was stopped and the fatty acids were extracted and counted for radioactivity in a LKB Rack β-counter as detailed by Peterson *et al.* (1985). One munit of lipase activity represents 1 nmol of fatty acids released/min. All samples for measurement of LPL activity were kept on ice before processing and the incubation was carried out within 4 h of sampling.

Statistics

Student's *t* test was used. The data are expressed as means ± S.E.M.

RESULTS

In the first set of experiments we compared the effects of low-M_r and conventional heparin when administered intravenously to rats. As shown in Fig. 1, plasma LPL activity began to rise within the first 1 min after injection of either heparin. After injection of 0.65 mg of conventional heparin/kg body wt. (equivalent to 100 units/kg) the LPL activity peaked between 3 and 10 min and then declined again. After 60 min the activity was close to the pre-heparin level, which was around 6 munits/ml in this study. With the same mass of low-M_r heparin the peak of LPL activity was reached earlier (around 3 min) and the peak value was only half of that reached with conventional heparin (272 versus 533 munits/ml; $P < 0.01$). When a higher dose (3.25 mg/kg) of conventional heparin was injected, close to maximal LPL activity was reached by 10 min and the activity then remained essentially unchanged up to 60 min, the longest time studied. The same dose of low-M_r heparin also gave peak activity by 10 min, but the activity was lower than with conventional heparin (497 versus 726 munits/ml; $P < 0.05$), and the activity did not remain at this level but decreased to less than one-third by 60 min. These findings are consistent with what has been reported by other investigators (Etienne *et al.*, 1983; de

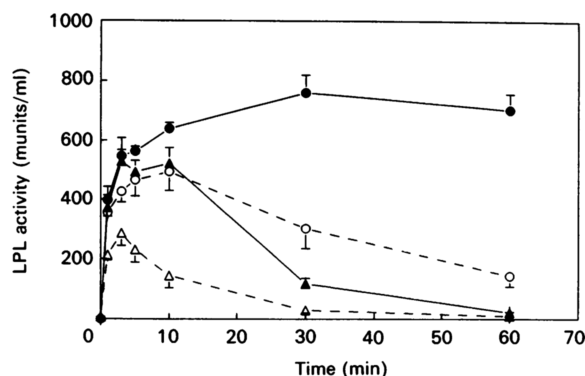

Fig. 1. Effects of intravenous administration of conventional and low-M_r heparins on plasma LPL activity in rats

Plasma LPL activity is shown after injection of conventional heparin (0.65 mg/kg; ▲); low-M_r heparin (0.65 mg/kg; △); conventional heparin (3.25 mg/kg; ●); and low-M_r heparin (3.25 mg/kg; ○).

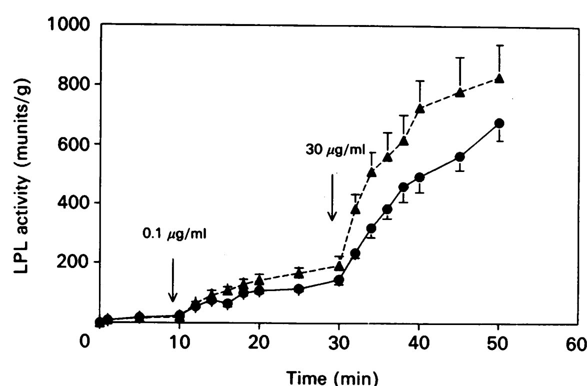

Fig. 2. Effects of conventional and low-M_r heparins on release of LPL activity from perfused rat hearts

The heart was stabilized by preperfusion for 10 min. Then, enough heparin was added to the reservoir to give a final concentration of 0.1 μg/ml in the perfusate and the perfusion was continued for 20 min in recirculating mode. More heparin was then added to the reservoir to raise the heparin concentration to 30 μg/ml and the perfusion was continued for another 20 min. Samples of the perfusate were taken at the indicated times for immediate assay of LPL activity. On each day, one heart was perfused with conventional heparin (●) and another heart was perfused with low-M_r heparin (▲). Values are means ± S.E.M. of three animals in each group, i.e. pooled from three experimental days.

Swart *et al.*, 1984; Persson *et al.*, 1985, 1987); i.e. low-M_r heparin appeared to have a lower ability to induce and maintain high plasma LPL activity in intact animals.

We then investigated whether more LPL remained at endothelial sites in the presence of low-M_r heparin. To explore this, we first administered the low dose (0.65 mg/kg) of either heparin. Then, 30 min later, the high dose (3.25 mg/kg) of conventional heparin was injected to release the LPL that had remained at endothelial sites. Table 1 shows that about the same LPL activity (net increase) was released in both groups. This suggests that low-M_r and conventional heparins had removed similar amounts of LPL from the endothelial sites during the first 30 min period, and that some mechanism other than a difference in LPL release was responsible for the lower plasma LPL activity 30 min after injection of low-M_r heparin.

In the next set of experiments we compared the effects of the two heparins in a heart perfusion system. Here, low-M_r heparin was at least as potent as conventional heparin in releasing LPL activity into the medium. Fig. 2 shows that when small amounts of the heparins (0.1 μg/ml) were added, a slow continuous release of LPL activity into the perfusate ensued. The activity released by low-M_r heparin slightly exceeded that with conventional heparin. At a higher heparin concentration (30 μg/ml) the release of LPL activity was more rapid; again, the effect of low-M_r heparin exceeded that of conventional heparin at each time point during the 20 min period studied (Fig. 2).

The above experiments suggested that low-M_r heparin had an ability similar to that of conventional heparin to release LPL. It is known that LPL is cleared by the liver, and that heparin slows down this process (Wallinder *et al.*, 1979; Peterson *et al.*, 1985; Chajek-Shaul *et al.*, 1988*a*; Vilaró *et al.*, 1988). To explore the role of the liver in determining the different plasma LPL activities after low-M_r compared with conventional heparin, we used the supradiaphragmatic rat model described by Bezman-Tarcher & Robinson (1965). In this system both heparins released similar amounts of LPL activity into the blood (Fig. 3). During the first

Table 1. Effect of injection of a high dose of conventional heparin on plasma LPL activity in rats which had previously received a low dose of either conventional or low-M_r heparin 30 min earlier

Rats were anaesthetized and given 0.65 mg of either conventional or low-M_r heparin/kg body wt. intravenously (first injection). At 5, 15 and 30 min later, small blood samples were taken for immediate assay of LPL activity. Then 3.25 mg of conventional heparin/kg body wt. was injected into all rats (second injection). Blood samples were taken 5, 15 and 30 min later (i.e. at 35, 45 and 60 min after the first injection). The net increase of LPL activity induced by this second injection was calculated by subtracting the LPL activity in the 30 min sample from those in the ensuing samples (35, 45 and 60 min). Values in parentheses are the net increases in LPL activity resulting from the second injection.

	LPL activity (munits/ml)					
	First injection			Second injection		
Type of heparin	5 min	15 min	30 min	35 min	45 min	60 min
Conventional	362±20	440±24	320±12	587±30 (267±31)	720±34	742±32
Low-M_r	181±10	118±8	47±7	337±28 (298±46)	463±26	522±33

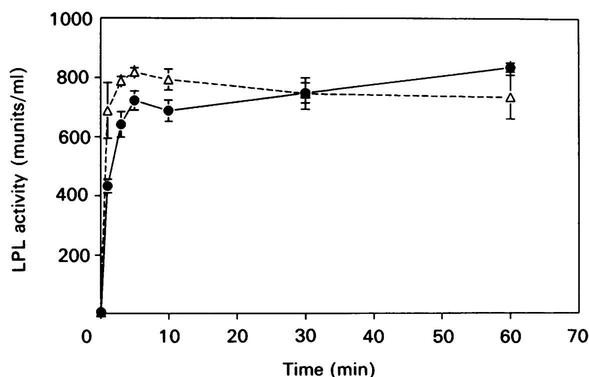

Fig. 3. Release of LPL activity by the two heparin preparations in supradiaphragmatic rats

Heparin was injected 10 min after the blood vessels below the diaphragm had been tied off. The dose was 1.1 mg/kg, i.e. one-third of the high dose used in Fig. 1. Rats were injected with either conventional heparin (●) or low-M_r heparin (△). Results are means ± S.E.M. of five animals in each group.

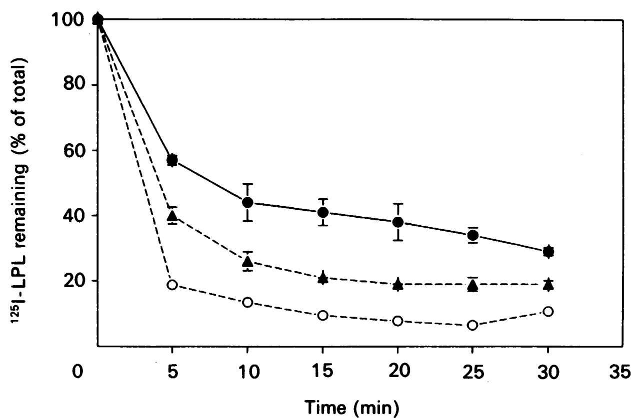

Fig. 4. Clearance of ^{125}I-labelled bovine LPL by the perfused rat liver

Livers were perfused for 10 min in recirculating mode. Then ^{125}I-LPL (about 50 ng; 10^6 c.p.m.) was added. When present, heparin was added 2 min before LPL to give a final concentration in the medium of 129 μg/ml. Values are expressed as percentages of initial radioactivity in the medium, and are means ± S.E.M.. ○, No heparin ($n = 2$); ●, conventional heparin ($n = 4$); ▲, low-M_r heparin ($n = 4$).

10 min the activities were actually higher with low-M_r than with conventional heparin, in accordance with the results of the heart perfusion experiments. The doses of both heparins in this experiment were one-third of the high dose used in Fig. 1, because the body mass left with intact circulation was about one-third of total body mass. In spite of this, higher LPL activities were reached than in the intact animals (compare Fig. 3 and Fig. 1).

To further study the role of the liver, we turned to a perfused liver system. ^{125}I-labelled bovine LPL added to the perfusate was rapidly cleared by the liver, in accordance with earlier studies (Vilaró *et al.*, 1988; Chajek-Shaul *et al.*, 1988*a*). Fig. 4 shows that both heparin preparations slowed down the rate of clearance. Low-M_r heparin was, however, substantially less efficient in this process than conventional heparin. Additional experiments showed that the clearance curve for exogenous LPL in the presence of a high concentration of low-M_r heparin (129 μg/ml) was close to the curve obtained with a low concentration of conventional heparin (6.5 μg/ml) (results not shown). When heparin was added to the perfusate 5 or 30 min after the addition of ^{125}I-LPL some, but not all, of the bound lipase returned to the perfusate (Fig. 5). Both heparins were equally effective in causing this release. The lipase radioactivity in the medium then decreased slowly. As observed in the experiments in which heparin was added before the lipase, the decrease in radioactivity in the medium occurred somewhat more slowly in the presence of conventional heparin than in the presence of low-M_r heparin.

To investigate whether there was a difference in the binding affinity for LPL between the two heparin preparations, they were fractionated by chromatography on LPL–Sepharose columns (Fig. 6). As previously described (Bengtsson *et al.*, 1980; Jackson *et al.*, 1985; Olivecrona & Bengtsson-Olivecrona, 1989), conventional heparin is heterogeneous with regard to binding to LPL. The preparation used here was eluted in the salt gradient as a broad peak between 0.1 and 0.7 M-NaCl. Low-M_r heparin showed a similar profile, but the peak was shifted to the left. At 0.4 M-NaCl, 53 % of the low-M_r preparation had been eluted as compared with only 36 % of the conventional heparin. This experiment showed that low-M_r heparin contained a higher proportion of molecules with low affinity for LPL and a lower proportion of molecules with high affinity for LPL than did the conventional heparin. One would therefore expect to see a difference in efficacy between the heparin preparations with respect to competition with other substances for binding of LPL.

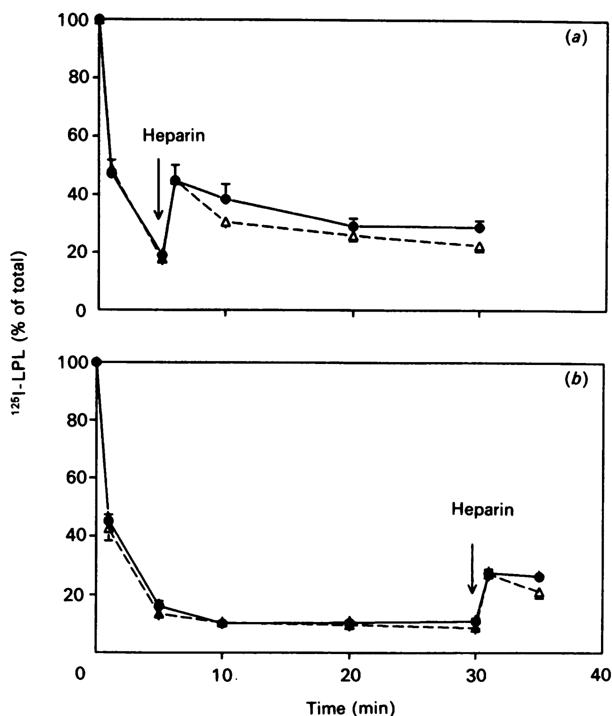

Fig. 5. Release of ^{125}I-labelled bovine LPL bound in the liver by addition of heparin to the perfusate

Livers were perfused with ^{125}I-LPL (about 50 ng; 10^6 c.p.m.) as in Fig. 4. At 5 min (*a*) or 30 min (*b*) after addition of the labelled lipase, conventional (●) or low-M_r (△) heparin was added to the perfusion medium to give a final concentration of 129 μg/ml. Values are percentages of initial radioactivity in the medium and are means ± S.E.M. of three experiments each.

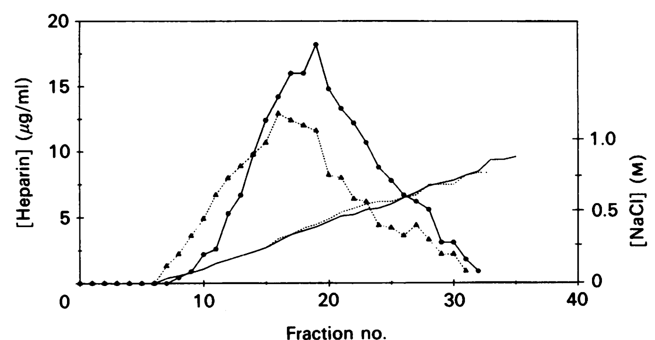

Fig. 6. Fractionation of the heparin preparations by affinity chromatography on immobilized LPL

LPL–Sepharose was packed in small columns which were equilibrated with 20 mM-Tris/HCl buffer, pH 7.4, containing 0.1 % Triton X-100. Conventional heparin (●) or low-M_r heparin (▲) was applied to the columns. In both cases the amount was 100 μg of heparin in 2 ml of buffer. The columns were washed with 10 ml of buffer and then eluted with gradients of NaCl of 0–1 M in the buffer (10 ml + 10 ml). The flow rate was 0.5 ml/min; fractions of 0.5 ml were collected. The salt concentration in the fractions was determined by conductometry. The solid line indicates the NaCl concentration during elution of conventional heparin; the broken line is NaCl concentration during elution of low-M_r heparin. The content of heparin in each fraction was determined spectrophotometrically in 250 μl portions.

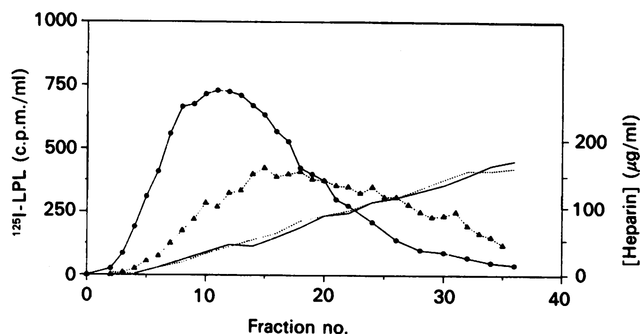

Fig. 7. Elution of LPL from heparin–Sepharose by gradients of heparin

LPL (2 ml; 10 µg/ml) in 10 mM-Tris/HCl, pH 7.4, containing 0.15 M-NaCl, 20% (w/v) glycerol and 0.1% (w/v) Triton X-100 was slowly passed through a small column containing 2 ml of heparin–Sepharose. A trace amount of ^{125}I-LPL was included in the sample to simplify detection. The columns were washed with 20 ml of the buffer and then eluted with a gradient of 0–200 µg of heparin/ml in the buffer (70 ml + 70 ml). Fractions of 4 ml were collected. Radioactivity was measured in 2 ml of each fraction and samples of 50 µl were used for chemical estimation of the amount of heparin. LPL radioactivity during elution with conventional heparin (●) and during elution with low-M_r heparin (▲) is shown. The heparin concentration during elution with a gradient of conventional heparin is shown by the solid line, and that during elution with low-M_r heparin by the broken line.

This was indeed evident when gradients of the two preparations were used to displace ^{125}I-labelled LPL from heparin–Sepharose (Fig. 7). The lipase started to detach from the heparin–Sepharose earlier with the gradient of conventional heparin and reached a peak at about 30 µg of heparin/ml, whereas with the low-M_r heparin the elution started later in the gradient and the peak was more extended.

DISCUSSION

Injection of the present preparation of low-M_r heparin resulted in lower plasma LPL activity than did injection of conventional heparin. This is in accordance with several previous studies (Etienne *et al.*, 1983; de Swart *et al.*, 1984; Persson *et al.*, 1985, 1987). The new aspect here is evidence that the main reason for the lower plasma LPL activity is that the low-M_r heparin is less effective in preventing hepatic clearance of released LPL from the circulation, in contrast with previous explanations (de Swart *et al.*, 1984; Persson *et al.*, 1985).

de Swart *et al.* (1984) were the first to report that low-M_r heparin results in lower levels of plasma LPL activity. They ascribed this to a lesser ability of the low-M_r preparation to release the enzyme from its endothelial binding sites. This was in accordance with the observation by Bengtsson *et al.* (1980) that short heparin fragments displayed lower affinity for LPL than did conventional heparin in a model system using polysaccharide-substituted agarose gels. Clarke *et al.* (1983) have shown that a heparin molecule with an M_r of 10000 or more can simultaneously bind to both heparin-binding sites on a dimeric LPL molecule. Whereas most heparin molecules in conventional heparin have M_r values above 10000, only about 10% of the molecules in the low-M_r heparin used here have such a high M_r. This means that most of the low-M_r heparin molecules can only bind to one site, and therefore form 2:1 heparin/LPL complexes with lower apparent affinity (Clarke *et al.*, 1983). The present studies *in vitro* showed that on average, the low-M_r heparin preparation displayed a lower affinity for LPL than did conventional heparin. Earlier studies have indicated that heparin preparations contain a mixture of molecules ranging from relatively low to relatively high affinity for LPL (Bengtsson *et al.*, 1980; Jackson *et al.*, 1985; Olivecrona & Bengtsson-Olivecrona, 1989). Both of the present preparations were clearly polydisperse in their interaction with LPL. Hence it is likely that the difference between the two preparations is that they have different profiles within a continuous spectrum of affinities for LPL. This is in contrast with the bimodal distribution of heparin molecules with respect to binding to antithrombin 3 (Höök *et al.*, 1976). In fact, results on plasma levels of LPL after injection of low-M_r heparin preparations have varied. Most studies report lower levels than obtained with conventional heparin. Harenberg *et al.* (1985), on the other hand, found similar levels. This has been discussed by Barrowcliffe (1986), who pointed out that the method of preparation of the low-M_r heparin used by Harenberg *et al.* (1985) involved a resulphation step, which results in a higher sulphate content than that of conventional heparins or other low-M_r heparins. It is likely that the affinity for LPL is directly related to the sulphate (and hence charge) content of heparin preparations (Olivecrona & Bengtsson-Olivecrona, 1989). The importance of the sulphate content for plasma LPL levels is illustrated by a study by Williams & Barrowcliffe (1985) in which two polysaccharides of low M_r, but high sulphate content, gave higher LPL levels than either unfractionated heparin or a low-M_r heparin with normal sulphate content. Thus increased sulphation seems to be able to compensate for the decrease in LPL affinity with decreased M_r.

Post-heparin plasma LPL activity reflects not only the release but also the clearance and inactivation of the enzyme (Olivecrona & Bengtsson-Olivecrona, 1989). To study directly the ability of the heparins to release the lipase from endothelial binding sites, we used a perfused heart model in which inactivation could be directly monitored and in which hepatic clearance was eliminated. The results showed that the low-M_r heparin released LPL at least as effectively as did conventional heparin. Evidence that the low-M_r heparin also had the same effect as conventional heparin in releasing LPL in intact animals was obtained in experiments in which a second injection of heparin was given to rats which had received a low dose of low-M_r or conventional heparin 30 min previously. The same net increase in LPL activity resulted from the second injection in both groups, which indicates that the two heparins had released similar amounts on the first injection, although with low-M_r heparin much of this released LPL had been cleared. Thus a lower capacity to release LPL is not a sufficient explanation for the lower plasma LPL activity induced by low-M_r heparin, regardless of the findings on binding affinity *in vitro*. Low-M_r heparin itself is cleared less rapidly than conventional heparin from the circulation (Bratt *et al.*, 1986; Dawes *et al.*, 1986; Palm & Mattsson, 1987; Mätzsch *et al.*, 1987); hence, a more rapid decrease in circulating heparin cannot explain the lower plasma LPL activity.

The other aspect of plasma LPL turnover is rapid clearing of the enzyme by the liver. The supradiaphragmatic rat proved to be a suitable model for elucidating the liver's role. In this model, plasma LPL activity reached equally high values after injection of low-M_r or conventional heparin, and did not decrease rapidly. This strongly indicates that the liver is responsible for the more rapid decrease in plasma LPL activity seen with low-M_r heparin in intact animals. The studies with ^{125}I-labelled bovine LPL in the liver perfusion model showed that low-M_r heparin was not as effective as conventional heparin in preventing LPL uptake in the dose range studied. It is not easy to relate these data to clearance *in vivo*; for that, arterio-venous difference studies in intact rats would be needed. It is apparent, however, that plasma LPL

activity after heparin injection does not represent a static equilibrium between lipase–heparin complexes in blood and lipase at endothelial binding sites, but rather a dynamic state with continuous flow of lipase molecules from peripheral tissues to the liver. Low-M_r heparin affects this flow in a quantitatively different way compared with conventional heparin: our data predict an initially similar release of lipase but then more clearance by the liver. In this respect, it is interesting to note a study by Persson *et al.* (1985), who infused conventional or low-M_r heparin together with Intralipid into normal volunteers. Initially both heparins caused increased clearance of the triacylglycerols and generation of high levels of non-esterified fatty acids. Later, however, triacylglycerol clearance slowed down in the individuals given low-M_r heparin, and actually became slower than before heparin was given. This observation is in accordance with the view that low-M_r heparin actually causes a loss in total LPL activity available for lipoprotein metabolism (i.e. at vascular endothelial sites or in the circulating blood).

It has been proposed that there are two types of binding sites for LPL in the liver (Vilaró *et al.*, 1988; Chajek-Shaul *et al.*, 1988*a*). One type of site is ligand-releasable by heparin and resembles the binding sites in extrahepatic tissues. The other type of binding site ('heparin-insensitive') is less sensitive to heparin and is found only (or at least mainly) in the liver (Vilaró *et al.*, 1988). These authors reported that both types of sites bound about equal amounts of LPL in liver perfusion. On the other hand, Chajek-Shaul *et al.* (1988*a*) have suggested that binding is initially mainly to the heparin-sensitive sites, and that the lipase then transfers to the other binding sites, which mediate internalization of the enzyme, unfolding with loss of catalytic activity, and ultimately degradation. In our experiments the perfused liver removed more than 80% of the ^{125}I-labelled LPL within 5 min, but only 25–30% could be released again by heparin; 15–20% was still available for release by heparin after 30 min. These data, which are in agreement with the results of Vilaró *et al.* (1988), suggest that at least half of the labelled LPL was immediately taken up by the heparin-insensitive sites and that transfer from heparin-sensitive to heparin-insensitive sites is relatively slow. In our study the low-M_r heparin released exogenous LPL from the liver to the same extent as did conventional heparin, implying that both heparins compete equally well with the heparin-sensitive sites in the liver. This would be analogous with the situation in peripheral tissues. The faster disappearance of ^{125}I-labelled LPL in the presence of low-M_r heparin therefore indicates that the heparin-insensitive binding sites may be responsible for the different clearance of LPL. The mechanisms by which heparin impedes hepatic uptake of LPL are, however, not fully understood. We do not know if the lesser effect of low-M_r heparin reflects the general features of these molecules, or if particular species of heparin molecules are responsible for the retardation of hepatic clearance. Further studies with more defined size and charge fractions of the low-M_r heparin preparation could resolve this. Such studies may also help to elucidate the detailed mechanisms of LPL uptake and metabolism in the liver.

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