Intravenous heparinase inhibits remnant lipoprotein clearance from the plasma and uptake by the liver: in vivo role of heparan sulfate proteoglycans

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Abstract Heparan sulfate proteoglycans (HSPG) are involved in the binding and uptake of apolipoprotein (apo) E-enriched remnant lipoproteins by cultured cells in vitro. To define the role of hepatic HSPG in remnant lipoprotein clearance in vivo, heparinase (30 units) was infused intravenously into mice to hydrolyze the liver HSPG and determine the effect of HSPG hydrolysis on remnant clearance by the liver. Liver HSPG were prelabeled by peritoneal injection of [^{35}S]Na_2SO_4. Injection of heparinase decreased the amount of ^{35}S-labeled liver HSPG by ~20–40% within 10–15 min. Heparinase infusion significantly inhibited the clearance of chylomicrons, chylomicron remnants, chylomicron remnants + apoE, rabbit β-very low density lipoproteins (β-VLDL), and β-VLDL + apoE. Compared with saline injection in control mice, heparinase injection retarded the plasma clearance of the remnants by 1.5- to 2-fold and decreased liver uptake by 1.3- to 1.6-fold. Confocal fluorescence microscopy of thick slices of liver from mice injected with 1,1'-dioctadecyl-3,3,3', 3'-tetramethylindocarbocyanine-labeled β-VLDL + apoE revealed markedly less intense fluorescence from hepatocytes in heparinase-treated animals compared with those in saline-treated control animals. Intravenous heparinase infusion did not inhibit the clearance of mouse low density lipoproteins (LDL), a ligand for the LDL receptor, and did not affect the clearance of α_2-macroglobulin, a ligand for the LDL receptor-related protein. The results suggest an important role of the liver HSPG in remnant clearance in vivo.—**Ji, Z-S., D. A. Sanan, and R. W. Mahley.** Intravenous heparinase inhibits remnant lipoprotein clearance from the plasma and uptake by the liver: in vivo role of heparan sulfate proteoglycans. *J. Lipid Res.* 1995. **36:** 583–592.

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Apolipoprotein (apo) E is an M_r = 34,000 glycoprotein that serves as a ligand for the low density lipoprotein (LDL) receptor and also interacts with heparin and heparan sulfate proteoglycans (HSPG) (1). It plays a key role in chylomicron remnant and very low density lipoprotein (VLDL) remnant clearance from the plasma (1–3). It is now envisioned that remnant lipoprotein binding and internalization by liver parenchymal cells involves several steps (4, 5). The initial clearance of these lipoproteins from the plasma (sequestration step) appears to involve their interaction with cell-surface HSPG, which are abundant in the space of Disse (6). The remnant lipoproteins are thought to become enriched in apoE, which is secreted by hepatocytes and is abundant in the space of Disse (7, 8). It has been shown that apoE-enriched remnants are cleared from the plasma rapidly in vivo (9) and that these remnants bind avidly to HSPG (4, 5) and to the LDL receptor-related protein (LRP) on ligand blots (10, 11). It is postulated that the apoE-enriched remnants are initially bound to the HSPG and subsequently internalized by the LRP after the remnants are transferred to the receptor or internalized by the HSPG–LRP complex.

Several studies have implicated cell-surface HSPG in the binding of remnant lipoproteins to cultured cells mediated by apoE (4, 5) or by lipoprotein lipase (12–15). Furthermore, 80% or more of the enhanced binding of β-VLDL remnants + apoE3 can be abolished by heparinase treatment of a variety of cells and most of the residual binding can be abolished by an anti-LDL receptor antibody that blocks ligand–receptor interaction (4). In addition, Chinese hamster ovary (CHO) cells (pgsD-677) lacking HSPG do not show the enhanced binding of remnant lipoproteins, whereas wild-type CHO cells avidly bind and internalize the apoE-enriched remnants. The enhanced binding of the apoE-enriched remnants to the wild-type CHO cells, which express both the LDL receptor and the LRP, is mediated primarily by HSPG; the residual binding is to the LDL receptor (4).

Abbreviations: apo, apolipoprotein; β-VLDL, β-very low density lipoproteins; CHO, Chinese hamster ovary; DiI, 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine; HSPG, heparan sulfate proteoglycans; LDL, Low density lipoproteins; LRP, LDL receptor-related protein.

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These studies have established clearly that cell-surface HSPG play a critical role in initiating binding and facilitating internalization of remnant lipoproteins in cell culture in vitro.

In addition to the involvement of the LDL receptor in the uptake of remnant lipoproteins (16), the LRP also appears to be involved (10, 11, 17–20). The LRP interacts with multiple ligands, e.g., α_2-macroglobulin (19, 21–23), apoE-enriched lipoproteins (10, 11, 18, 19), lipoprotein lipase (24, 25), lactoferrin (24), various proteases (24, 26–29), and *Pseudomonas* exotoxin A (30). These ligands are all internalized by the liver and degraded (for review, see ref. 31). The present studies were undertaken to attempt to establish an in vivo role for HSPG in remnant lipoprotein metabolism. Intravenous injection of heparinase into the general circulation or directly into the liver through the portal vein significantly inhibited the plasma clearance and liver uptake of remnant lipoproteins but did not alter the uptake of activated α_2-macroglobulin clearance by the LRP or the uptake of LDL by the LDL receptor. These studies support the concept that HSPG are involved in vivo in remnant lipoprotein recognition and uptake.

MATERIALS AND METHODS

Preparation of lipoproteins

Radiolabeled canine chylomicrons and chylomicron remnants were obtained from normal adult mongrel dogs (~45–65 lb), as described previously (9). The chylomicron remnants were prepared in functionally hepatectomized rabbits that were injected intravenously with [^{3}H]retinol-labeled canine chylomicrons (200 mg of triglyceride/kg of body weight) and then were isolated from the plasma of the rabbits after 20 min of circulation. Chylomicrons and chylomicron remnants were centrifuged and washed twice by overlaying the plasma with a d 1.006 g/ml solution. Rabbit β-VLDL were prepared from the plasma of New Zealand White rabbits fed a high-fat, high-cholesterol diet for 4 days, as described by Kowal et al. (10). The β-VLDL were centrifuged twice at d 1.006 g/ml and labeled with iodine by the method of Bilheimer, Eisenberg, and Levy (32) or with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine (DiI), a fluorescent dye, by the procedure described (4). Mouse LDL (d 1.019–1.04 g/ml) were isolated from the plasma of ICR-strain males by ultracentrifugation (33).

Enrichment of the remnants in apoE

Remnant lipoproteins were enriched in purified human apoE3 (provided by Dr. Karl H. Weisgraber) by incubating them together at 37°C for 1 h prior to use (34). A ratio of remnant lipoprotein protein to human apoE3 of 1:1.5 was used (4).

Other reagents

The α_2-macroglobulin (CALBIOCHEM Corp., La Jolla, CA) was activated with methylamine as described (19). Bovine lactoferrin was purchased from Sigma Chemical Co. (St. Louis, MO). Activated α_2-macroglobulin and lactoferrin were labeled with IODO-GEN (Pierce Chemical Co., Rockford, IL). Free iodine was removed with a PD10 column (Pierce Chemical Co.).

Heparinase (EC 4.2.2.7) (Sigma, Heparinase I, catalog number H 2519) was solubilized in sterile 0.15 M NaCl before use. The enzymatic activity was assayed by the method of Khan and Newman (35).

Biochemical procedures

The protein concentration was determined by the method of Lowry et al. (36). The concentrations of triglyceride and cholesterol were measured with a Spectrum High Performance Diagnostic System using aqueous standards (Abbott Laboratories, North Chicago, IL).

Labeling of hepatic HSPG with [^{35}S]sulfate

The [^{35}S]Na_2SO_4 (ICN Biochemicals, Cleveland, OH) was used to label mouse liver HSPG as previously described (4). Briefly, 250 μCi of [^{35}S]Na_2SO_4 was injected intraperitoneally into each mouse (ICR, ~30 g). To assess HSPG labeling after 2 or 16 h, mice were injected with either 30 units of heparinase in 100 μl of saline or with 100 μl of sterile saline (0.15 M NaCl) alone via the tail vein or portal vein. Fifteen min after injection of the heparinase or saline, the mice were anesthetized, and the livers were perfused with cold phosphate-buffered saline, pH 7.4, through the portal vein. After exsanguination, two pieces of liver from each animal were necropsied and solubilized in Soluene 350 tissue solubilizer (Packard Instrument Company, Inc., Downers Grove, IL), and the [^{35}S]sulfate was measured by scintillation counting and the amount of [^{35}S]sulfate (cpm/g of liver) was determined. The level of [^{35}S]sulfate in the saline-injected controls was set at 100%. The percent released by heparinase was calculated by comparison with the values in the control animals.

Isolation of mouse liver HSPG

Mice (ICR, ~30 g) were injected intraperitoneally with 250 μCi of [^{35}S]Na_2SO_4 and anesthetized 2 h later. The portal vein was exposed and injected with heparinase (30 units) or 0.15 M NaCl. Fifteen min later the livers were perfused with phosphate-buffered saline. Two g of liver was homogenized and used for isolation of the liver matrix HSPG as described (37, 38). Briefly, after homogenization of the liver and centrifugation, the pellet was extracted with 2 M NaCl. The supernatant was diluted to 0.2 M NaCl with a buffer containing 8 M urea, 50 mM sodium acetate (pH 6.0), and 0.5% Triton X-100, and the matrix HSPG was isolated on a Sepharose Q

column. The ^{35}S-labeled proteoglycans were eluted from the column using a 0.2–1.0 M NaCl gradient. Fractions (1 ml) were collected and quantitated by scintillation counting. The isolated ^{35}S-labeled proteoglycans were identified as HSPG by their susceptibility to hydrolysis with heparinase (4, 38). Briefly, the column fractions containing the proteoglycans were run on 3–20% sodium dodecyl sulfate-polyacrylamide gels, transferred to nitrocellulose membranes, and incubated with or without heparinase. The membranes were subjected to autoradiography (4).

Remnant lipoprotein clearance after heparinase or saline injection

Varying amounts of heparinase (in 100 μl of 0.15 M NaCl) or 0.15 M sterile NaCl (100 μl) were injected slowly into the tail vein or portal vein of ICR mice (~30 g) through a 24-gauge needle. (The level of heparinase varied as indicated in the specific figure legends but was usually 30 units.) The mice injected with heparinase through the tail vein were kept in an unanesthetized condition during the experimental procedure. The mice injected with heparinase via the portal vein were first anesthetized with pentobarbital (~0.3 mg/mouse) in 100 μl of saline that was injected into the peritoneal cavity. The portal vein was exposed, and heparinase was injected through a resident catheter. Anesthesia was maintained throughout the surgical and experimental procedures. Typically, the heparinase was allowed to circulate for 5 min prior to the injection of the radiolabeled lipoproteins, activated α_2-macroglobulin, or lactoferrin. The amount of radiolabeled material injected is indicated for each study, as is the time that it circulated before the animals were killed and a liver sample was taken for quantitation of the level of uptake of the radiolabeled macromolecules. Plasma clearance and hepatic uptake were calculated on the basis of the percent of the injected dose of labeled material at different times after its injection. A plasma volume of 3.5% of body weight was used for the calculations.

Hepatic uptake of DiI-labeled β-VLDL + apoE

Mice were anesthetized with Metafane (Pitman-Moore, Inc., Mundelein, IL) in a closed bell jar. Anesthesia was maintained throughout the surgical procedures and experimental circulation times by means of a nose cone containing a Metafane-impregnated cotton pad. The portal vein was exposed by laparotomy. By means of a resident cannula and tubing with a 50 μl dead volume, 30 units of heparinase in 50 μl of physiological saline was infused over a period of 1 min into the portal vein. Control mice were infused with the same volume of saline without heparinase. After 5 min of circulation, the DiI-labeled β-VLDL + apoE (10 μg β-VLDL protein + 15 μg apoE in 50 μl saline) were injected into the portal vein. The mice were carefully covered to minimize desiccation and kept warm by means of a heating pad. Ten min after injection, the animals were killed and their livers were fixed by perfusion with 3% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) after an initial flush with buffer alone. Intact lobes or slices of livers were examined by fluorescence confocal microscopy (Bio-Rad MRC-600, Bio-Rad Laboratories, Hercules, CA).

RESULTS

These studies were designed to determine the effect of intravenous heparinase on uptake of various ligands by the mouse liver. The initial studies demonstrated that hepatic HSPG could be released by intravenous heparinase. Then these studies were extended to the effects of intravenous heparinase on plasma clearance and liver uptake of lipoproteins and other macromolecules.

Release of [^{35}S]sulfate from the livers of mice after intravenous heparinase

The HSPG in the mouse liver were labeled for 2 h or 16 h by intraperitoneal injection of [^{35}S]Na_2SO_4, and then the ability of intravenous heparinase to release the [^{35}S]sulfate from the liver was determined as described under Materials and Methods. As shown in **Fig. 1,** heparinase (30 units) was injected either into the tail vein or portal vein and the amount of [^{35}S]sulfate released was measured at 10, 15, or 30 min after the injection. Approximately 20–40% of the [^{35}S]sulfate was released from the liver at 10 or 15 min. The greatest release occurred 15 min

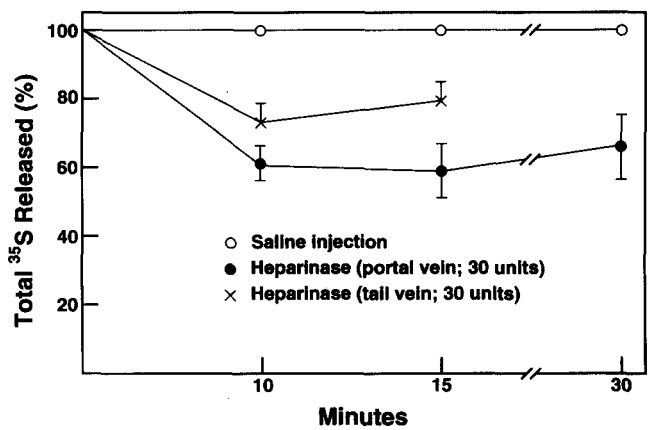

Fig. 1. Effect of intravenous heparinase versus saline on the release of [^{35}S]sulfate from the mouse liver. Hepatic HSPG were prelabeled with [^{35}S]Na_2SO_4 for 2 h prior to the injection of 0.15 M NaCl (saline, 100 μl) (○) or heparinase (30 units in 100 μl of saline) via the tail vein (x) or the portal vein (●). The livers were perfused at the indicated times with 0.1 M phosphate-buffered saline, liver samples (0.2–0.4 g) were solubilized, and [^{35}S]sulfate was quantitated by scintillation counting as described in Materials and Methods. The percent of the total ^{35}S released is based on a comparison of the level of activity in the saline-injected controls, which was set at 100%. The mean ± SD was calculated from results obtained with three to six mice at each time point.

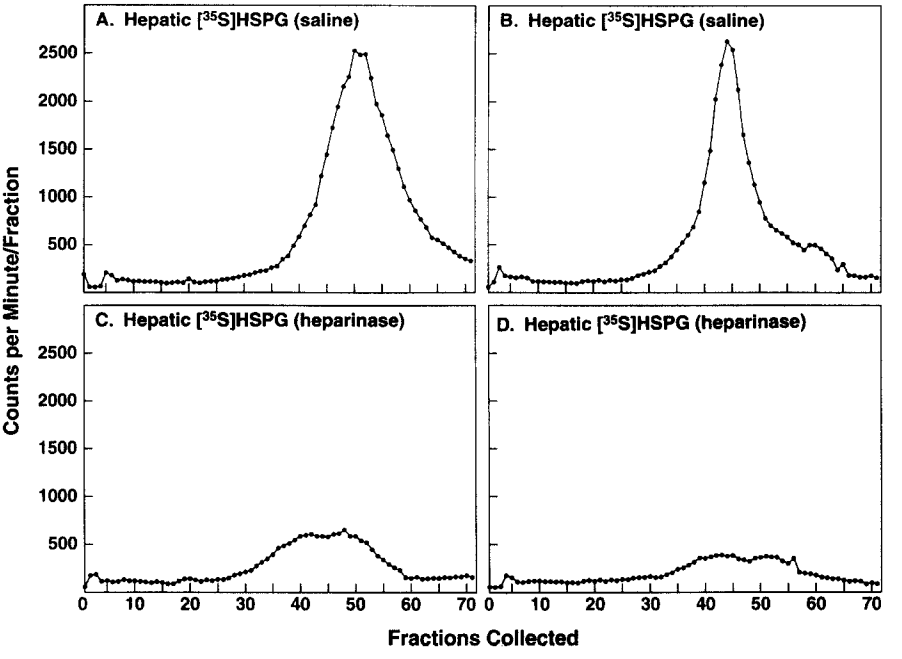

Fig. 2. Effect of intravenous heparinase versus saline on the amount of ^{35}S-labeled HSPG that could be isolated from mouse liver. A and B: [^{35}S]HSPG isolated from the livers of two mice infused via the portal vein with 0.15 M NaCl (saline). C and D: [^{35}S]HSPG isolated from the livers of two mice infused via the portal vein with heparinase (30 units). The HSPG were isolated from homogenized liver, extracted with 2 M NaCl, and subjected to column chromatography to obtain matrix HSPG, as described in Materials and Methods. Fractions (1 ml) were collected and the [^{35}S]sulfate was quantitated by scintillation counting.

after portal vein injection of heparinase (~40% release). In this series of experiments, heparinase or saline was injected 2 h after the intraperitoneal administration of the [^{35}S]Na_2SO_4. In a separate series of experiments, the heparinase was injected into the portal vein 16 h after the intraperitoneal [^{35}S]Na_2SO_4. In these studies the percent of [^{35}S]sulfate released after heparinase injection was 29% at 15 min.

In addition, it was established that HSPG were reduced in the mouse liver after heparinase injection. As shown in **Fig. 2,** the amount of [^{35}S]HSPG in the liver of mice injected intravenously with heparinase was markedly reduced compared to the amount of [^{35}S]HSPG isolated from saline-injected mice. Therefore, intravenous infusion of heparinase not only rapidly decreases the ^{35}S label in the liver, but also decreases the amount of [^{35}S]HSPG in the liver.

Concentration of heparinase required for optimal inhibition of plasma clearance and liver uptake of β-VLDL + apoE

Intravenous injection of heparinase inhibited β-VLDL + apoE clearance and uptake. Various concentrations of heparinase (10–100 units) were injected and allowed to circulate for 5 min. Then the ^{125}I-labeled β-VLDL + apoE were injected, and 10 min after lipoprotein injection (15 min after heparinase injection) the plasma level and liver uptake of the lipoproteins were determined. As will be shown in the next section, the action of the intravenous heparinase was rapid, and 5 min of circulation of the heparinase prior to lipoprotein injection was an adequate time interval for an optimal effect.

As shown in **Fig. 3,** both routes of administration of

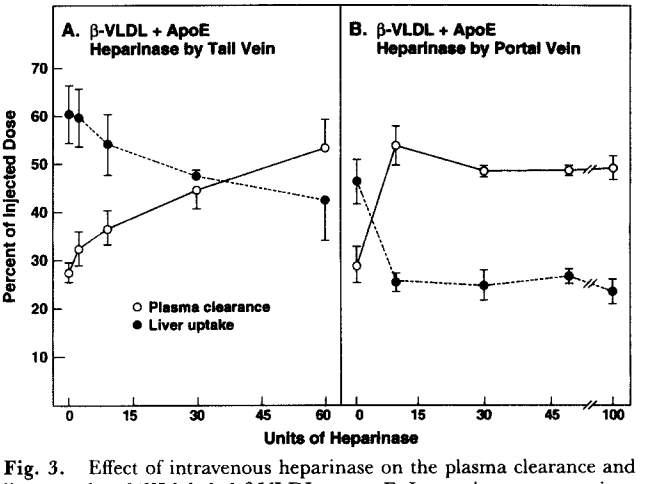

Fig. 3. Effect of intravenous heparinase on the plasma clearance and liver uptake of ^{125}I-labeled β-VLDL + apoE. Increasing concentrations of heparinase were injected into the tail vein (panel A) or the portal vein (panel B) and allowed to circulate for 5 min. The mice were then injected with ^{125}I-labeled β-VLDL + apoE (5 μg of β-VLDL protein + 7.5 μg of apoE) and killed 10 min later. The plasma clearance (○) and the liver uptake (●) are presented as a percent of the injected dose remaining in the plasma or taken up by the liver (the data represent the mean ± SD from three mice at each concentration of heparinase).

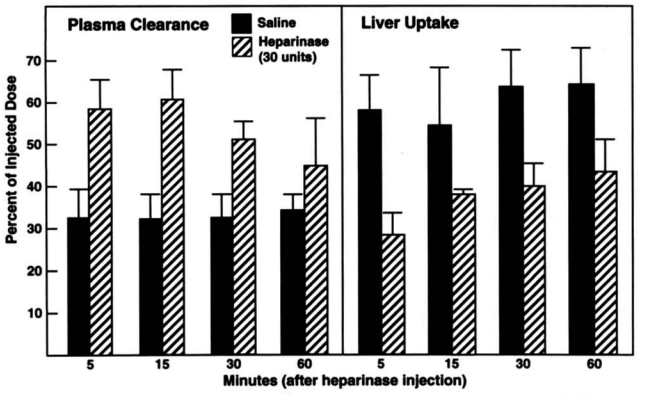

Fig. 4. Effect of time of heparinase or saline circulation on the plasma clearance and liver uptake of ^{125}I-labeled β-VLDL + apoE. Heparinase (30 units) or saline was injected into the tail vein and allowed to circulate for 5, 15, 30, or 60 min before the ^{125}I-labeled β-VLDL + apoE (5 μg of β-VLDL protein + 7.5 μg of apoE) were injected. The mice were killed 10 min later and the plasma clearance and liver uptake were determined as described in Materials and Methods. The data are presented as percentages of the injected dose remaining in the plasma or taken up by the liver (mean ± SD, n = 3) at each time point.

heparinase inhibited clearance and uptake of the β-VLDL + apoE; however, a somewhat more pronounced effect occurred when the heparinase was injected via the portal vein. As can be seen in Fig. 3A, a larger dose of heparinase was required to obtain the maximal effect when it was injected into the tail vein, whereas lower doses could be used when it was injected through the portal vein (Fig. 3B). In the studies to follow, 30 units of heparinase were used unless otherwise specified.

Optimal circulation time of heparinase prior to the injection of β-VLDL + apoE

Heparinase (30 units) was allowed to circulate for 5, 15, 30, or 60 min prior to the injection of the ^{125}I-labeled β-VLDL + apoE. The control animals received saline instead of heparinase. The plasma clearance and liver uptake of the β-VLDL + apoE were then determined at 10 min after injection of the lipoproteins. As shown in **Fig. 4,** the effect of the intravenous injection of heparinase on plasma clearance and liver uptake was rapid. At 5 min after injection of heparinase, maximal inhibition of both plasma clearance and hepatic uptake occurred. At 60 min after heparinase injection, the inhibition of uptake was still pronounced; however, the trend was toward less inhibition. In the studies to follow the heparinase was allowed to circulate for 5 min before injection of the tracer dose of the molecule to be studied.

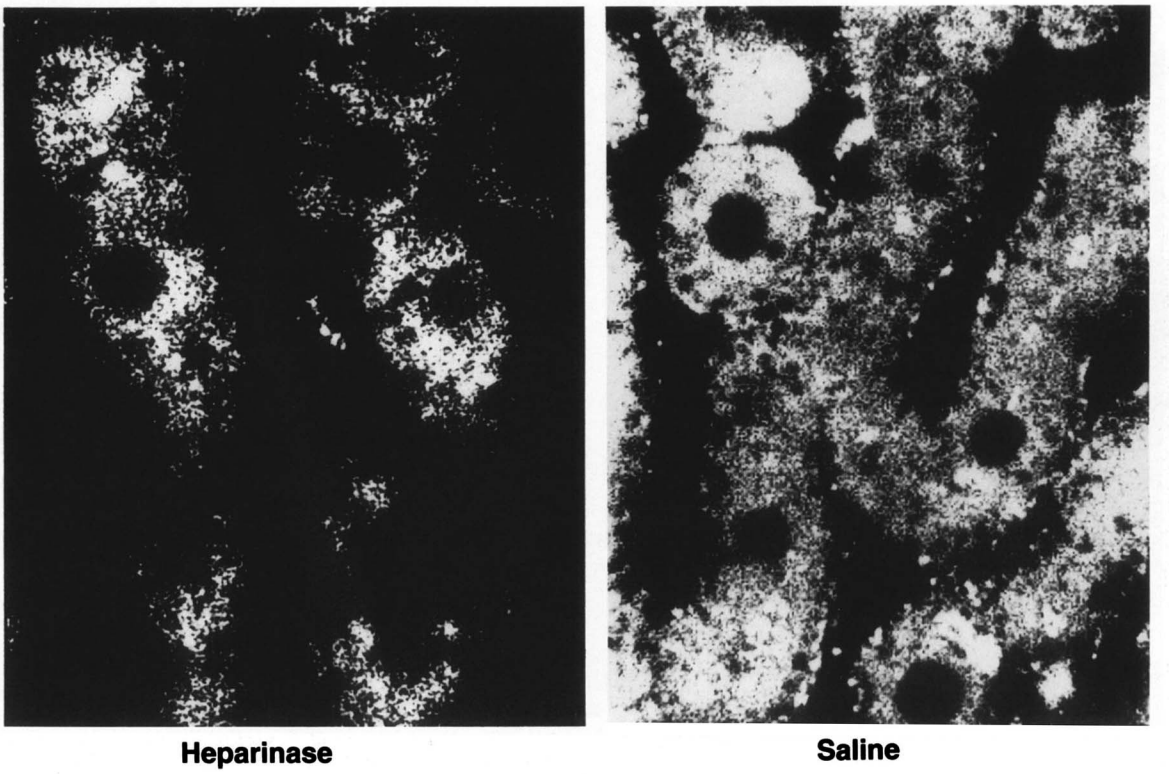

Fig. 5. Effect of heparinase or saline on the uptake of DiI-labeled β-VLDL + apoE by the liver. There was a marked uptake of the fluorescently labeled remnant lipoproteins by the hepatocytes of the saline-infused liver (right panel). By comparison, infusion of heparinase (30 units) very significantly decreased the level of uptake (left panel). The heparinase and saline, as well as the DiI-labeled β-VLDL + apoE (10 μg of β-VLDL protein + 15 μg of apoE), were infused into the portal vein. The heparinase and saline were allowed to circulate for 5 min, and then the lipoproteins circulated for 10 min prior to killing the animals and perfusion fixation of the livers as described in Materials and Methods. In each micrograph, cords of hepatocytes containing fluorescence are seen. The large spherical nuclei (5–6 in each section), which are not fluorescently labeled, allow identification of the location of the hepatocytes. Magnification, × 978.

Effect of intravenous heparinase on hepatocyte uptake of DiI-labeled β-VLDL

The intense fluorescence in hepatocytes of the saline-injected controls indicated a marked uptake of DiI-labeled β-VLDL at 10 min after the injection of lipoproteins (**Fig. 5**). Intravenous injection of heparinase (30 units) via the portal vein resulted in a very significant reduction in the uptake of the fluorescently labeled β-VLDL (Fig. 5). There was a low fluorescence signal both at the sinusoidal front and within the hepatocytes in heparinase-treated livers when compared with saline-treated control livers. Therefore, the fluorescence confocal microscopy suggests that heparinase infusion inhibits both sequestration and internalization in the mouse liver.

Effect of heparinase administered by the tail vein on plasma clearance and liver uptake of various lipoproteins and α_2-macroglobulin

As shown in **Table 1,** α_2-macroglobulin, a ligand for the LRP, was cleared from the plasma and taken up by the liver identically in the saline- and heparinase-injected mice. On the other hand, heparinase injection significantly retarded the plasma clearance and liver uptake of canine chylomicrons, chylomicron remnants with and without added apoE, and β-VLDL with and without added apoE.

The chylomicrons and chylomicron remnants displayed very similar values for clearance and uptake in the control mice and a similar inhibition by heparinase (Table 1). However, the addition of apoE to the chylomicron remnants very markedly accelerated their clearance from the plasma and uptake by the liver. Nevertheless, heparinase injection significantly inhibited liver uptake. On the other hand, the clearance of β-VLDL enriched in apoE was similar to that of the β-VLDL without added apoE. We assume that in these in vivo studies the β-VLDL already have sufficient apoE or that they acquire additional apoE from the plasma to mediate enhanced binding. Alternatively, in vivo the space of Disse may contain enough apoE to enhance the binding of β-VLDL, but not of chylomicron remnants. However, regardless of whether added apoE stimulated β-VLDL clearance or not, heparinase retarded the clearance and uptake 1.5- to 2-fold.

Effect of heparinase administration by the portal vein on plasma clearance and liver uptake of various lipoproteins, α_2-macroglobulin, and lactoferrin

As shown in **Fig. 6,** infusion of heparinase into the portal vein of mice markedly increased the percentage of the injected dose of the β-VLDL + apoE3 retained in the

TABLE 1. Effect of heparinase (administered by tail-vein injection) on the plasma clearance and liver uptake of various lipoproteins and α_2-macroglobulin

	Saline Control		Heparinase Injection	
	Plasma Clearance[a]	Liver Uptake[a]	Plasma Clearance[a]	Liver Uptake[a]
	percent of injected dose of labeled molecules			
α_2-Macroglobulin (activated)	22.3 ± 5.8 (n = 20)	59.5 ± 12.1	21.3 ± 6.5 (n = 21)	63.8 ± 10.5
Chylomicrons	32.3 ± 6.6 (n = 12)	44.8 ± 8.8	49.0 ± 8.1[b] (n = 12)	34.1 ± 6.2[b]
Chylomicron remnants	31.3 ± 8.4 (n = 12)	47.0 ± 9.8	47.5 ± 6.8[b] (n = 12)	31.1 ± 4.3[b]
Chylomicron remnants + apoE	9.3 ± 2.2 (n = 10)	56.6 ± 7.1	18.4 ± 3.5[b] (n = 8)	41.9 ± 5.6[b]
β-VLDL	23.3 ± 3.9 (n = 15)	53.9 ± 5.8	45.4 ± 6.1[b] (n = 16)	34.7 ± 5.3[b]
β-VLDL + apoE	28.3 ± 2.3 (n = 6)	55.0 ± 4.7	42.8 ± 3.1[b] (n = 6)	34.6 ± 4.3[b]

In all studies, heparinase (30 units) was allowed to circulate for 5 min before injection of the radiolabeled tracer molecule. However, in each case, the time after injection of the α_2-macroglobulin or the various lipoproteins at which the plasma clearance and liver uptake were determined varied as follows: chylomicrons, 15 min; chylomicron remnants, chylomicron remnants + apoE, β-VLDL, and β-VLDL + apoE, 10 min; and α_2-macroglobulin, 5 min. In addition, various numbers of individual preparations of the lipoproteins and α_2-macroglobulin were used in the different studies as follows: chylomicrons, three different preparations; chylomicron remnants, three different preparations; β-VLDL, five different preparations; and α_2-macroglobulin, four different preparations. The amounts of lipoprotein or α_2-macroglobulin injected were as follows: ^{3}H-labeled chylomicrons, 5 mg of triglyceride/mouse; ^{3}H-labeled chylomicron remnants, 5 mg of triglyceride/mouse; ^{3}H-labeled chylomicron remnants + apoE, 1.5 mg of triglyceride + 30 μg of apoE/mouse; ^{125}I-labeled β-VLDL, 5 μg of protein/mouse; ^{125}I-labeled β-VLDL + apoE, 5 μg of β-VLDL protein + 7.5 μg of apoE/mouse; and ^{125}I-labeled α_2-macroglobulin, 2 μg/mouse.

[a] The plasma clearance and liver uptake are presented as a percent of the injected dose remaining in the plasma or taken up by the liver, respectively.

[b] The significance determined between plasma clearance and liver uptake in saline controls versus heparinase-injected mice was $P < 0.001$ (Student's t test).

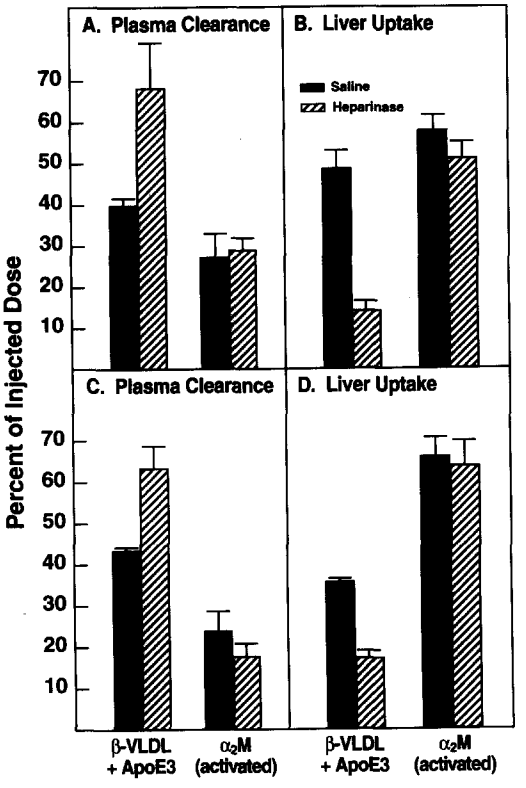

Fig. 6. Effect of portal vein infusion of heparinase versus saline on plasma clearance and liver uptake of β-VLDL + apoE and activated α_2-macroglobulin (α_2M). Heparinase (30 units) or saline was infused slowly into the portal vein and allowed to circulate for 5 min. Then the clearance and uptake of the ^{125}I-labeled β-VLDL + apoE (5 μg of β-VLDL protein + 7.5 μg of apoE) and α_2-macroglobulin (2 μg) were determined at 10 min and 5 min, respectively. Heparinase decreased the plasma clearance and hepatic uptake of the ^{125}I-labeled β-VLDL + apoE but had no significant effect on the clearance and uptake of activated α_2-macroglobulin. The results from two independent studies are shown (A-B and C-D) and are presented as the mean ± SD for three mice injected with β-VLDL + apoE3 or with α_2-macroglobulin in each study.

plasma. Furthermore, heparinase decreased the amount of β-VLDL + apoE3 taken up by the liver to 13.4% of the injected dose compared to 47.5% of the dose taken up in the saline-injected control mice 10 min after injection (Figs. 6A and B). These are among the most dramatic results that were observed. In a separate study performed using a different preparation of β-VLDL, heparinase decreased the amount of β-VLDL + apoE3 taken up by the liver to 16.9%, versus 35.5% in the saline-injected control mice (Figs. 6C and D). In each of these studies α_2-macroglobulin clearance and uptake were found to be essentially identical in the saline- and heparinase-injected mice (Fig. 6).

The data for several independent studies in numerous mice are summarized in **Table 2.** No significant difference was observed between the plasma clearance and liver uptake of α_2-macroglobulin and LDL in the control (saline-injected) versus the heparinase-injected mice. Thus, heparinase injection does not appear to alter the function of the LRP or the LDL receptor. Likewise, it has been shown previously that heparinase does not affect the LRP or the LDL receptor in cultured cells or on ligand blots (4). However, the injection of heparinase into the portal vein exerted a very large effect on plasma clearance and liver uptake of the β-VLDL + apoE (Table 2). Liver uptake was reduced from 48% of the injected dose in the control mice to 22% after heparinase injection (2.1-fold inhibition). On the other hand, the percent of the injected dose of the β-VLDL + apoE remaining in the plasma of the control mice was 38% compared to 65.5% following heparinase injection (a factor of 1.75).

Lactoferrin, which binds to the LRP (24) and is cleared rapidly by the liver (39, 40), was used as a control to attempt to demonstrate that at least part of the clearance of this molecule was mediated by liver HSPG and could be inhibited by heparinase. We have demonstrated in cultured cells that lactoferrin binds to both HSPG and the LRP (41). As shown in Table 2, ^{125}I-labeled lactoferrin was readily taken up by the liver in the control mice (81% of the injected dose at 5 min). However, after the injection of heparinase, the uptake was markedly reduced (65.5% of the injected dose). The amount remaining in the plasma of the control animals at 5 min was 4.5% compared to 18.7% in the heparinase-injected mice.

DISCUSSION

Cell culture studies have strongly implicated cell-surface HSPG in the initial binding of remnant lipoproteins and suggested that cell-surface HSPG are essential for receptor-mediated uptake in vitro, presumably involving the LRP (4, 5). Apolipoprotein E has been shown to be critical for the binding and uptake of remnant lipoproteins in a number of studies (for review, see refs. 1 and 2). In addition, lipoprotein lipase has been implicated in increasing the binding of remnant lipoproteins to cells, possibly by facilitating lipoprotein interaction with the LRP (25) or with HSPG (14). Furthermore, it has been reported that lipoprotein lipase induces the catabolism of several different lipoproteins, e.g., VLDL, LDL, and lipoprotein[a], by first facilitating their binding to cell-surface HSPG (12–15, 42, 43). Likewise, it has been suggested that hepatic lipase participates in remnant clearance (44); it also has been shown to mediate enhanced binding of remnant lipoproteins to cell-surface HSPG (45, 46). Therefore, the initial rapid step in plasma clearance of remnants, envisioned as a sequestration of the lipoproteins in the space of Disse, has been postulated to involve remnant lipoprotein–HSPG interaction, which subsequently could facilitate the uptake of the particles by

TABLE 2. Effect of heparinase (administered by portal-vein injection) on the plasma clearance and liver uptake of various lipoproteins, α_2-macroglobulin, and lactoferrin

	Saline Control		Heparinase Injection	
	Plasma Clearance	Liver Uptake	Plasma Clearance	Liver Uptake
	percent of injected dose of labeled molecules			
α_2-Macroglobulin (activated)	28.1 ± 5.5 (n = 3)	59.3 ± 4.1	29.3 ± 3.0 (n = 3)	51.0 ± 4.6
LDL	73.6 ± 3.3 (n = 3)	17.9 ± 7.0	77.7 ± 6.0 (n = 3)	15.3 ± 4.4
β-VLDL + apoE	38.1 ± 9.4 (n = 12)	48.1 ± 6.5	65.5 ± 11.8[a] (n = 12)	22.3 ± 7.4[a]
Lactoferrin	4.5 ± 0.7 (n = 9)	81.0 ± 10.6	18.7 ± 6.2[a] (n = 8)	65.5 ± 5.7[b]

In all studies, heparinase (30 units) was allowed to circulate for 5 min before injection of the radiolabeled tracer molecule. However, the time after injection of the α_2-macroglobulin, lactoferrin, or the various lipoproteins at which the plasma clearance and liver uptake were determined varied as follows: α_2-macroglobulin, 5 min; lactoferrin, 5 min; LDL, 15 min; and β-VLDL + apoE, 10 min. The plasma clearance and liver uptake are presented as a percent of the injected dose remaining in the plasma or taken up by the liver, respectively. In addition, various numbers of individual preparations of various molecules were used in the different studies as follows: α_2-macroglobulin, one preparation; lactoferrin, two different preparations; LDL, one preparation; β-VLDL + apoE, four different preparations. The amounts of lipoproteins, activated α_2-macroglobulin, and lactoferrin injected were as follows: ^{125}I-labeled α_2-macroglobulin, 2 μg/mouse; ^{125}I-labeled lactoferrin, 10 μg/mouse; ^{125}I-labeled LDL, 5 μg of protein/mouse; ^{125}I-labeled β-VLDL + apoE, 5 μg of β-VLDL protein + 7.5 μg of apoE/mouse.

[a]The significance determined between plasma clearance and liver uptake of saline controls versus heparinase-injected mice was $P < 0.001$ (Student's t test).

[b]The significance determined between liver uptake in saline controls versus heparinase-injected mice was $P < 0.01$ (Student's t test).

hepatocyte lipoprotein receptors (4, 5).

The present studies were undertaken to attempt to extend those in vitro observations to the in vivo situation. It is clearly acknowledged that the intravenous injection of heparinase, even when it is administered slowly via the portal vein, could have other effects in addition to the hydrolysis of hepatic HSPG. However, the consistency seen between the cell-culture studies and the impact of heparinase on the mouse liver further suggests the importance of HSPG and extends the in vitro observations related to remnant lipoprotein clearance. Furthermore, intravenous heparinase did not alter the clearance of LDL by the LDL receptor or of activated α_2-macroglobulin by the LRP. The intravenous injection of heparinase into mice resulted in a rapid release of ^{35}S label from liver prelabeled with [^{35}S]Na_2SO_4. Within 10–15 min of heparinase injection, ~20–40% of the ^{35}S-labeled material was released compared to the level of ^{35}S within the livers of saline-injected control animals. This phenomenon was observed with both 2-h and 16-h prelabeling of the hepatic proteins (primarily HSPG) by an intraperitoneal injection of [^{35}S]Na_2SO_4. In addition, much less [^{35}S]HSPG could be extracted and isolated from the liver after heparinase injection. Thus, heparinase infusion clearly reduces the level of hepatic HSPG.

Experimentally it was determined that precirculation of heparinase for 5 min and the injection of 30 units of heparinase was satisfactory to obtain near-optimal inhibition of plasma clearance and liver uptake of β-VLDL + apoE. Inhibition of remnant clearance was observed after the delivery of the heparinase to the general circulation via the tail vein or directly to the liver via the portal vein. The largest effects on remnant clearance, however, occurred after portal vein injection. For example, the plasma clearance and liver uptake of β-VLDL + apoE were typically reduced by 1.75- and 2-fold, respectively, after heparinase injection. In addition, a dramatic reduction in fluorescently labeled β-VLDL + apoE uptake by the livers of mice after heparinase treatment clearly confirmed the effect of the infusion of this enzyme on plasma clearance and liver uptake.

Therefore, we have demonstrated that the in vivo clearance and hepatic uptake of remnant lipoproteins (chylomicrons and β-VLDL) are very significantly inhibited by the action of intravenous heparinase. It is speculated that the release of ^{35}S label reflects the hydrolysis of cell-surface HSPG from the liver, including the HSPG of the space of Disse, and that the impaired clearance of the remnant lipoproteins reflects the importance of hepatic HSPG in this process in vivo. There are, of course, several possible ways by which HSPG hydrolysis could affect remnant clearance. For example, the heparinase could deplete the space of Disse of apoE by removing the sulfated HSPG and thus interfere with the initial sequestration step in remnant catabolism. However, the present studies suggest that in the absence

of HSPG after heparinase injection, the injection of excess apoE along with the remnant lipoproteins does not restore their clearance to the rapid rates seen in the saline-injected control mice. Therefore, it appears likely that one of the effects of heparinase could be to deplete the space of Disse of the HSPG essential for apoE-mediated enhanced clearance and uptake. In addition, heparinase could affect clearance by removing or interfering with the initial binding of lipoprotein lipase or hepatic lipase to the cell-surface HSPG. At the present time we cannot distinguish among these possibilities.

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