Hepatic Lipase Gene Therapy in Hepatic Lipase–deficient Mice

Adenovirus-mediated Replacement of a Lipolytic Enzyme to the Vascular Endothelium

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Abstract

Hepatic lipase (HL) is an endothelial-bound lipolytic enzyme which functions as a phospholipase as well as a triacylglycerol hydrolase and is necessary for the metabolism of IDL and HDL. To evaluate the feasibility of replacing an enzyme whose in vivo physiologic function depends on its localization on the vascular endothelium, we have infused recombinant replication-deficient adenovirus vectors expressing either human HL (HL-rAdV; $n = 7$) or luciferase cDNA (Lucif-rAdV; $n = 4$) into HL-deficient mice with pretreatment plasma cholesterol, phospholipid, and HDL cholesterol values of 176±9, 314±12, and 129±9, respectively. After infusion of HL-rAdV, HL could be detected in the postheparin plasma of HL-deficient mice by immunoblotting and postheparin plasma HL activities were 25,700±4,810 and 1,510±688 nmol/min/ml on days 5 and 15, respectively. Unlike the mouse HL, 97% of the newly synthesized human HL was heparin releasable, indicating that the human enzyme was virtually totally bound to the mouse vascular endothelium. Infusion of HL-rAdV in HL-deficient mice was associated with a 50–80% decrease in total cholesterol, triglyceride, phospholipids, cholesteryl ester, and HDL cholesterol ($P < 0.001$) as well as normalization of the plasma fast protein liquid chromatography lipoprotein profile by day 8. These studies demonstrate successful expression and delivery of a lipolytic enzyme to the vascular endothelium for ultimate correction of the HL gene defect in HL-deficient mice and indicate that recombinant adenovirus vectors may be useful in the replacement of endothelial-bound lipolytic enzymes in human lipolytic deficiency states. (*J. Clin. Invest.* 1996. 97:799–805.) Key words: gene therapy • Adenoviridae • hepatic lipase • HDL • phospholipid

Introduction

Hepatic lipase (HL)[1] is a 60-kD lipolytic enzyme that plays an important role in the hydrolysis of triglycerides and phospholipids present in circulating plasma lipoproteins. As both a triacylglycerol lipase and phospholipase, HL mediates the conversion of IDL to LDL and HDL_2 to HDL_3 (1–3). In the HDL process, HL may regenerate nascent pre-β HDL particles (4, 5) necessary for the removal of free cholesterol from peripheral cells, thus playing an important role in cholesterol homeostasis (6, 7) and ultimately enhancing the process of reverse cholesterol transport.

HL is synthesized and secreted primarily by parenchymal liver cells (8, 9). In humans and most vertebrates this lipolytic enzyme is almost entirely bound to the hepatic vascular endothelium via heparin-like glycosaminoglycans (10). In contrast, mouse HL has a relatively low affinity for heparin-like glycosaminoglycans and is found in circulating plasma presumably due to the lack of a high-affinity binding site (11). Although mouse HL is 86% homologous to the human enzyme there is a significant sequence divergence in the carboxy terminus which may be responsible for the observed differences in heparin-binding affinities of the two enzymes (12).

The important role that HL plays in lipid and lipoprotein metabolism has been established by the identification of patients with HL deficiency that present with marked dyslipidemia including hypertriglyceridemia, hypercholesterolemia, and accumulation of β-VLDL (13–15). Recently, the underlying molecular defects leading to HL deficiency in some of these kindreds have been reported (16–19). Affected individuals have increased plasma concentrations of HDL_2 as well as phosphatidylcholine enrichment of HDL (13), suggesting an important role of HL not only in LDL but also in HDL metabolism. In addition, at least a subset of patients with HL deficiency appears to be at an increased risk for developing premature coronary artery disease (13, 20).

The metabolic consequences of overexpressing the HL gene in different animals models have also been investigated. Overexpression of the human HL gene in transgenic rabbits results in a fivefold decrease in plasma cholesterol concentration and a reduction in IDL (21). In addition, transgenic mice that overexpress the human HL gene appear to have reduced cholesterol accumulation in the aorta during a hyperlipidemic diet (22), suggesting that at least in these animals increased HL activity may result in relative protection against atherosclerosis.

Recently, an animal model for human HL deficiency, the HL-deficient mouse, has been reported (23). Unlike the human dyslipoproteinemia, HL-deficient mice do not appear to develop marked hypertriglyceridemia or accumulation of β-VLDL. However, like patients with HL deficiency, HL-deficient mice have increased plasma concentrations of cholesterol and phospholipids attributable to increased HDL levels.

In this study, we use this HL-deficient animal model to evaluate the feasibility of replacing an endothelial-bound lipolytic enzyme, human HL, using recombinant adenovirus

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Received for publication 18 August 1995 and accepted in revised form 7 November 1995.

1. *Abbreviations used in this paper:* FPLC, fast protein liquid chromatography; HL, hepatic lipase.

J. Clin. Invest.

0021-9738/96/02/0799/07 $2.00
Volume 97, Number 3, February 1996, 799–805

vectors. The studies reported here indicate that the human HL gene can be successfully delivered to the liver for synthesis and ultimate binding to the vascular endothelium where it can function physiologically to normalize the abnormal lipid profile present in HL-deficient mice.

Methods

Animals. The HL-deficient mice used in these studies have been described previously (23). All animals were adult males between 2 and 3 mo old, ~ 25–30 grams in weight, and were fed a regular chow diet (NIH-07 chow diet 5% fat; Zeigler Brothers, Inc., Gardners, PA).

Generation of recombinant adenovirus. The recombinant adenoviruses HL-rAdV and Lucif-rAdV, containing the human HL cDNA (24, 25) or the firefly luciferase (26, 27), respectively, were constructed as described by McGrory et al. (28). Briefly, pAd12-HL, a pXCX2 plasmid derived from pXC1 (29) containing the CMV promoter and enhancer elements, the SV40 splice donor, acceptor and polyadenylation signal as well as the HL cDNA inserted into the E1 region of the human adenovirus (AdV5) genome, was cotransfected with pJM17 using the method of Chen and Okayama (28, 30). pAd12-Lucif was similarly constructed and cotransfected. Recombinant adenoviruses were identified by PCR as well as by the presence of either HL or luciferase activity in the media and subjected to two rounds of plaque purification before large scale amplification in human embryonal kidney 293 cells (American Type Culture Collection, Rockville, MD) (31). Infected cells were harvested 48–72 h after infection and subjected to five cycles of freeze/thaw lysis. Crude lysates were extracted with Freon (Halocarbon 113; Matheson Gas Products, Secaucus, NJ), banded twice in CsCl, supplemented with 0.2% mouse albumin (Sigma Chemical Co., St. Louis, MO), and dialyzed extensively against 150 mM NaCl, 10 mM Hepes (pH 7.4), 5 mM KCl, 1 mM $MgCl_2$, and 1 mM $CaCl_2$. Recombinant adenovirus was stored at −70°C and titered (32) before infusion into the animals. The absence of contaminating wild-type adenovirus was confirmed by PCR screening using oligonucleotide primers located within the structural portion of the deleted Ela region (evaluating for the presence of AdV5 sequences 562 to 899 bps; GenBank accession number M73260). An appropriate volume (100–300 μl) of the purified recombinant adenovirus containing 10^8–10^9 plaque-forming units was infused into the saphenous vein of the mice on day 0 of the study.

Blood sampling. For all blood sampling, the mice were fasted for 4 h (water available) and anesthetized with methoxyflurane (33). Bleeding was performed from the retroorbital plexus using capillary tubes coated with heparin (Scientific Products, McGaw Park, IL). Blood samples were placed into precooled tubes containing 0.2 M EDTA (final concentration 4 mM) and kept on ice until centrifuged at 2,500 *g* for 20 min. Plasma was removed, aliquoted, immediately frozen in dry ice, and stored at −70°C.

Lipid and lipoprotein measurements. Fasting mouse plasma (10 μl) was diluted 1:50 with PBS and lipids were measured by enzymic assays using commercially available kits: total cholesterol (Sigma Diagnostics, St. Louis, MO); free cholesterol and phospholipids (Wako Chemicals USA, Inc., Richmond, VA); and the Cobas Mira Plus automated chemistry analyzer (Roche Diagnostic Systems, Inc., Branchburg, NJ). Cholesteryl ester was calculated as the difference between total cholesterol and free cholesterol. HDL cholesterol was determined as the cholesterol remaining in the plasma after precipitation of the apolipoprotein B–containing lipoproteins with heparin and calcium as described previously (34).

Fast protein liquid chromatography (FPLC). Plasma lipoproteins were separated by gel filtration using two Superose 6 HR 10/30 columns connected in series (Pharmacia Biotech Inc., Piscataway, NJ) (35). Lipoproteins from 50 μl of plasma were eluted at 0.3 ml/min with PBS buffer containing 1 mM EDTA and 0.02% sodium azide. Lipids in the recovered fractions were quantitated without the dilution described above. Lipoprotein elution volumes were: VLDL, 15.0–16.0 ml; IDL/LDL, 20.0–24.0 ml; HDL, 30.0–31.0 ml. Plasmas from at least three separate animals for each group were analyzed by FPLC.

Lipase assays. To obtain postheparin plasma, animals were anesthetized by intraperitoneal injection with 0.011 ml/gram of animal weight using 2.5% Avertin prepared by dissolving 10 grams tribromoethanol in 10 ml tertiary amyl alcohol. Anesthetized animals were injected with heparin (500 U/Kg) through the tail vein using a 30-gauge needle. Blood was sampled 5 min after the heparin injection as described above. HL activity was assayed by incubation with a radiolabeled triolein substrate in the presence of 1 M NaCl with no additional lipoprotein activator (36). Each assay tube contained 150 μl of sonicated substrate (1.4 μM glycerol trioleate, Sigma Chemical Co.; 0.063 μCi glycerol tri-[1-^{14}C]-oleate, Amersham Corp., Arlington Heights, IL; 150 μg phosphatidylcholine, Sigma Chemical Co.; 0.075 M NaCl; 0.112 M Tris-HCl buffer, pH 8.5; and 8.5% BSA), 100 μl 5 M NaCl, the appropriate volume of pre- or postheparin mouse plasma, and 0.15 M NaCl to a total volume of 500 μl. The volume of mouse plasma (0.5–10 μl) was adjusted as necessary to maintain linearity of the assay.

Western blots. Before electrophoresis, mouse postheparin plasma (100 μl pooled from three mice) was bound to heparin-Sepharose CL-6B (600 μl, Pharmacia Biotech Inc.) equilibrated with 0.01 M sodium phosphate buffer, pH 7.6. The resin and postheparin plasma were gently rotated for 30 min at 7°C. Unbound protein (including albumin) was removed by thorough washing with the equilibration buffer. Elution of bound proteins was performed stepwise with elution buffer containing 0.4 M NaCl, 0.8 M NaCl, and 1.5 M NaCl. The eluate was dialyzed into 0.01 M NH_4HCO_3 and dried on a Savant SpeedVac concentrator (Savant Instruments, Inc., Farmingdale, NY). The dried samples were resuspended in a minimal volume of sample buffer, separated in a 10% Tris-glycine gel (Novex, San Diego, CA), and transferred to polyvinylidene difluoride microporous membranes (Immobilon PVDF; Millipore, Bedford, MA) as described previously (37). Human HL was identified by blotting with a goat polyclonal antibody (kindly provided by Dr. Ira Goldberg, Columbia University, New York) and visualized by silver-enhanced detection of a gold-labeled rabbit anti–goat antibody (Auroprobe, Amersham Corp.). Protein standards of known molecular weight (SeeBlue, San Diego, CA) and HL standard were used on each blot. Human HL standard was isolated from media obtained from 293 cells transfected with HL cDNA under the control of the CMV promoter/enhancer (37).

Luciferase assays. Tissues were obtained 4 d after mice were infused with Lucif-rAdV. The animals were killed by cervical dislocation, and their organs were removed immediately and frozen in dry ice. The tissues were homogenized in extraction buffer (100 mg in 0.5 ml; 0.1 M potassium phosphate buffer, pH 7.4, and 1 mM DTT) with a hand-held homogenizer (Omni International, Inc., Marietta, GA). After three freeze/thaw cycles, the homogenates were centrifuged (10,000 *g*, 20 min, 4°C) and the supernatants were removed for assay. The supernatant (30 μl) was then incubated with luciferin in the presence of 330 μl of reaction mixture (16.4 mM $MgCl_2$ and 5.4 mM ATP), the resulting relative light units determined with the Monolight luminometer (Analytic Luminescence Laboratory, San Diego, CA) (38).

Data analysis. Data are presented as the mean±SEM. Comparisons between groups were made using the *t* test and paired comparisons were performed with the paired *t* test using Excel 5 (Microsoft, Redmond, WA).

Results

Table I summarizes the plasma lipids, lipoproteins, and postheparin HL activity values for control and HL-deficient mice used in the study. Compared with age- and sex-matched control animals, HL-deficient mice had 1.5–2-fold higher plasma concentrations of total cholesterol, phospholipids, cholesteryl

Table I. Plasma Lipids, Lipoproteins, and Lipase Activity in Control and the HL-deficient Mice

	TC	TG	PL	CE	HDL-C	HL activity	
						Preheparin	Postheparin
			mg/dl			*nmol/min/ml*	
HL-deficient ($n = 7$)	176±9*	54±4	314±12*	122±8*	129±9*	5±5	10±2
Controls ($n = 13$)	101±2	63±2	211±4	66±2	78±3‡	130±1166	257±32§

*$P < 0.001$; ‡ $n = 10$; and § $n = 6$. *TC*, total cholesterol; *TG*, triglycerides; *PL*, phospholipids; *CE*, cholesteryl ester; and *HDL-C*, HDL cholesterol.

ester, and HDL cholesterol ($P < 0.001$) but similar levels of fasting plasma triglycerides. HDL cholesterol represented 80% of the total cholesterol for both groups of mice. Previous studies (11) have demonstrated that ~ 50% of normal mouse HL activity is present in plasma before heparin infusion. Consistent with these earlier findings, the age- and sex-matched control animals used in the current study had significant HL activity present in both pre- and postheparin plasma (Table I) indicating the presence of circulating, unbound active mouse HL. As described previously (23), HL-deficient mice had virtually no detectable HL activity in either pre- or postheparin plasma, indicating an absolute deficiency of the enzyme.

The replication-deficient recombinant adenoviruses generated for these studies are illustrated in Fig. 1. These vectors contained expression cassettes consisting of either the human HL cDNA (HL-rAdV) or luciferase cDNA (Lucif-rAdV) under the control of the CMV promoter and enhancer with an SV40 splice donor and acceptor as well as an SV40 polyadenylation signal sequence.

Previous studies (39, 40) have demonstrated that systemic infusions of recombinant adenovirus result in delivery of the transgene primarily to the liver. These findings were confirmed by quantitation of the relative luciferase units present in different tissues 4 d after infusion of Lucif-rAdV via the saphenous vein in mice. Thus, analysis of mouse liver, brain, heart, kidney, lung, spleen, and testes demonstrated expression of luciferase primarily in mouse liver (data not shown) indicating that when infused into the systemic circulation adenovirus appears to be hepatotrophic and is a suitable vector for the expression of hepatically derived proteins.

However, replacement of the HL gene in HL-deficient mice is a more complex process. In addition to the human HL gene being delivered to the liver for synthesis, posttranslational modification, and secretion, the mature human HL must also be transported to the capillary endothelium for binding to mouse endothelial glycosaminoglycans followed by hydrolysis of triglyceride and phospholipid present on circulating plasma lipoproteins by the active endothelial-bound HL (Fig. 2). To evaluate the feasibility of performing gene therapy for endothelial-bound lipolytic enzymes in lipase deficiency syndromes and other genetic dyslipoproteinemias, ~ 10^9 plaque-forming units of HL-rAdV or control Lucif-rAdV were delivered via saphenous vein infusion in HL-deficient mice. Gene expression was determined by quantitation of HL mass and activity in postheparin plasma. Immunoblot analysis of postheparin plasma isolated from HL-deficient mice before and after virus infusion demonstrated the presence of a major 60-kD immunoreactive band in the plasma of treated animals (Fig. 3) which was not detected in postheparin plasma of the same mice before HL-rAdV infusion. Thus, replacement of the HL gene was achieved in HL-deficient mice using adenovirus vectors.

Table II summarizes the HL activity in postheparin plasma of HL-deficient mice before and after infusion of either HL-rAdV or Lucif-rAdV. Compared with preinfusion values,

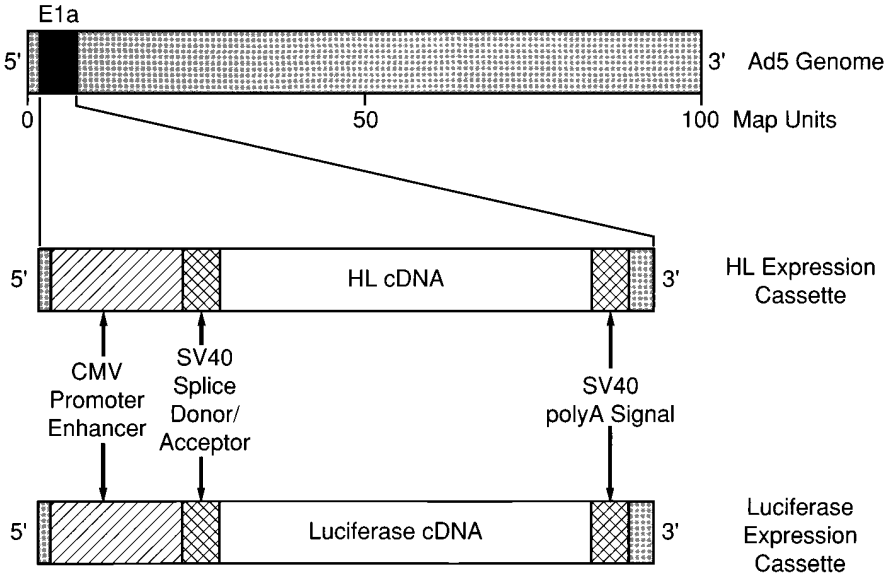

Figure 1. Recombinant adenovirus constructs. The expression cassettes contained either human HL cDNA or firefly luciferase cDNA under the control of the CMV promoter/enhancer as well as the SV40 splice site donor, acceptor, and polyadenylation signal which were inserted into the Ela/Elb region of the nonreplicative Ad5 genome by homologous recombination in 293 cells.

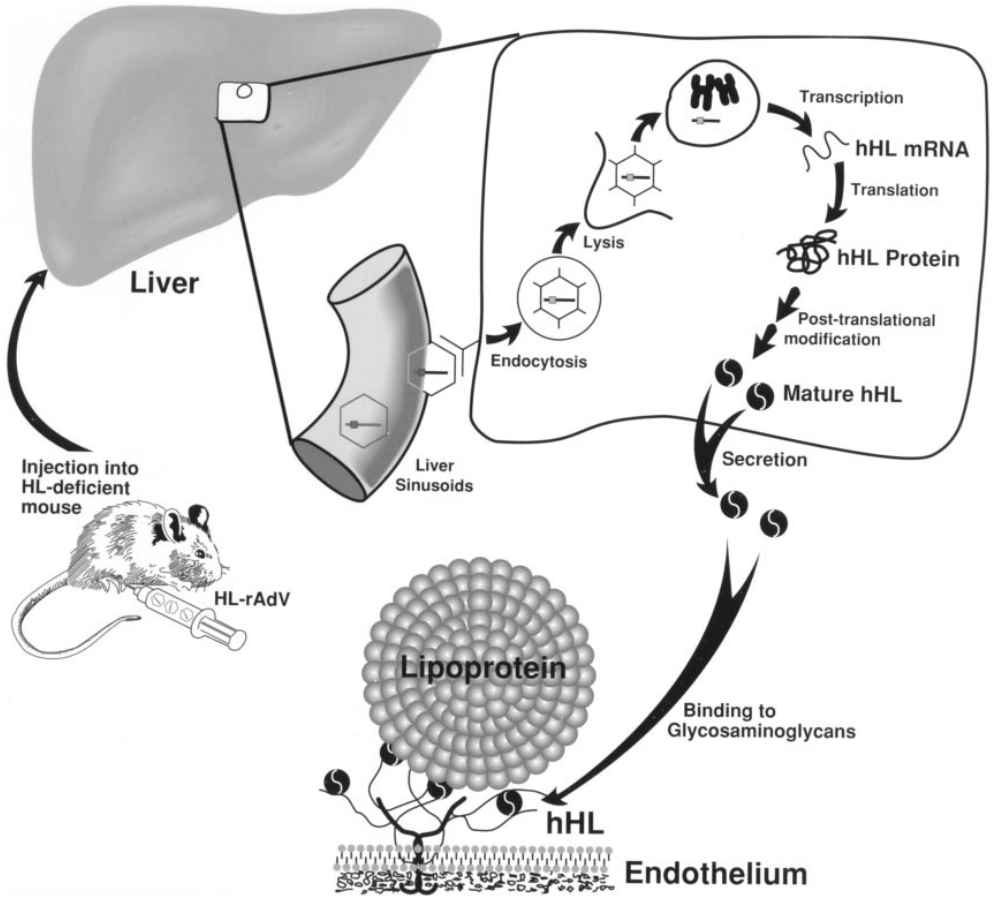

Figure 2. Schematic diagram for successful gene therapy of HL. Recombinant adenovirus is infused into the saphenous vein of the mice for delivery of the human HL gene to the liver. After synthesis, post-translational modification, and secretion, the mature human HL must be transported to the capillary endothelium for binding to mouse endothelial glycosaminoglycans. Hydrolysis of triglycerides and phospholipids present on circulating plasma lipoproteins by the active endothelial-bound HL results in particle remodeling.

postheparin plasma HL activity was increased significantly ($P < 0.05$) 5 and 15 d after HL-rAdV infusion. 30 d after injection with HL-rAdV, 50% of the treated HL-deficient mice demonstrated persistent HL activity. As expected, HL-deficient mice treated with Lucif-rAdV had no detectable increase in HL activity.

Table III summarizes the plasma lipids and lipoproteins in HL-deficient mice before and after virus infusion. HL gene replacement in HL-deficient mice resulted in an ~ 80% reduction of plasma cholesterol, phospholipids, and cholesteryl ester and a 50% reduction in plasma triglycerides by day 4. By 8 d after infusion with HL-rAdV, total cholesterol, triglycerides, and phospholipids as well as HDL cholesterol levels were normalized to control values.

FPLC profiles of plasma lipoproteins from control and HL-deficient mice before and after HL-rAdV infusion are illustrated in Fig. 4. Representative plasma FPLC profiles for both groups of animals indicate that the majority of cholesterol and phospholipids are present in HDL-sized lipoprotein particles. Compared with controls, HL-deficient mice have a relative enrichment of cholesterol and phospholipid resulting in larger-sized HDL. After infusion of HL-rAdV, the HDL cholesterol and phospholipids in HL-deficient mouse are decreased and the FPLC lipoprotein profiles of treated animals can be superim-

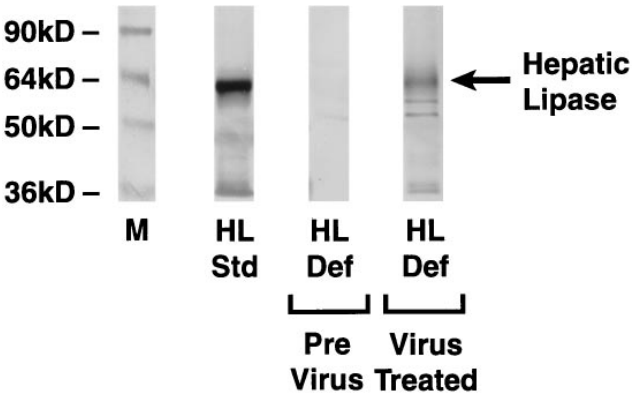

Figure 3. Immunoblot analysis of postheparin plasma from HL-deficient mouse infused with HL-rAdV. Molecular weight standards are shown on the left (*M*). HL standard was isolated from media of 293 cells transfected with a human HL expression vector. Postheparin plasma from HL-deficient mice before (*Pre Virus*) and after (*Virus Treated*) infusion was chromatographed on heparin-Sepharose. Proteins eluting in the 0.8 M NaCl fractions were analyzed by SDS-PAGE followed by immunoblotting with an HL monospecific antibody.

Table II. HL Activity in Postheparin Plasma of HL-deficient Mice after Infusion of HL-rAdV or Lucif-rAdV

	Postheparin plasma-HL activity		
	Day 0	Day 5	Day 15
	nmol/min/ml		
HL-rAdV ($n = 7$)	10±2	25700±4810*	1510±688‡
Lucif-rAdV ($n = 4$)	8±3	8±1	3±4

* $P < 0.001$; ‡ $P < 0.05$.

Table III. Plasma Lipids and Lipoproteins in HL-deficient Mice before and after Infusion of HL-rAdV

	TC	TG	PL	CE	HDL-C
			mg/dl		
Day 0 ($n = 7$)	176±9*	58±4	314±12*	122±8*	129±9*
Day 4 ($n = 7$)	35±6	31±11	73±10	13±6	21±4
Day 8 ($n = 5$)	94±10	51±5	207±16	35±12	62±8
Controls ($n = 13$)	101±2	63±2	211±4	66±2	78±3‡

*$P < 0.001$ versus control; ‡ $n = 10$. Abbreviations as in Table I.

posed on those of control mice. Thus, HL gene replacement using recombinant adenovirus resulted in normalization of the lipid composition as well as lipoprotein profile in HL-deficient mice.

The time course of the changes in the mean total cholesterol and phospholipid concentrations in the HL-deficient mice after HL-rAdV or Lucif-rAdV infusion is illustrated in Fig. 5. After infusion with HL-rAdV, there is a rapid decrease in cholesterol and phospholipid levels observed by day 2 followed by a gradual return to pretreatment values. In contrast, no significant changes in either cholesterol or phospholipid plasma concentrations were detected in animals infused with a similar dose of Lucif-rAdV, indicating that the reduction in plasma lipids in HL-rAdV–treated animals was a result of HL expression and not secondary to nonspecific viral-induced changes.

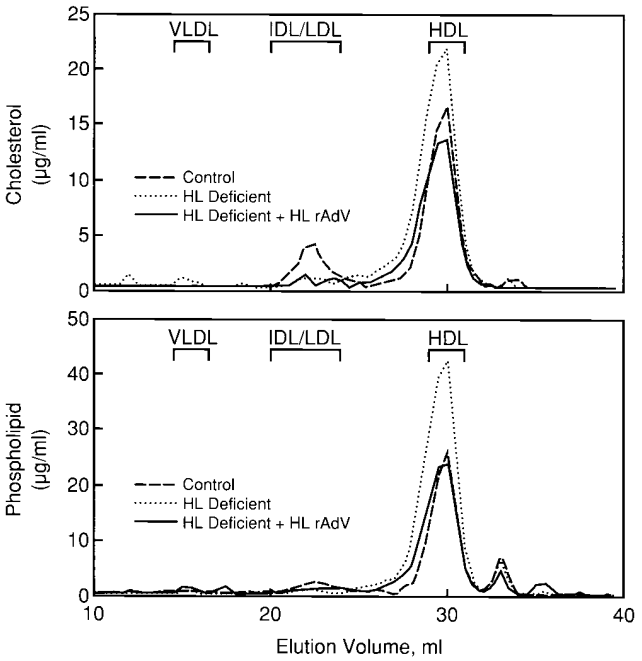

Figure 4. Representative FPLC profiles of plasma from control and HL-deficient mice separated on Superose 6 columns. The lipid profiles before (*dotted lines*) and after infusion with HL-rAdV (*solid lines*) are compared with those from control C57BL/6 mice (*dashed lines*).

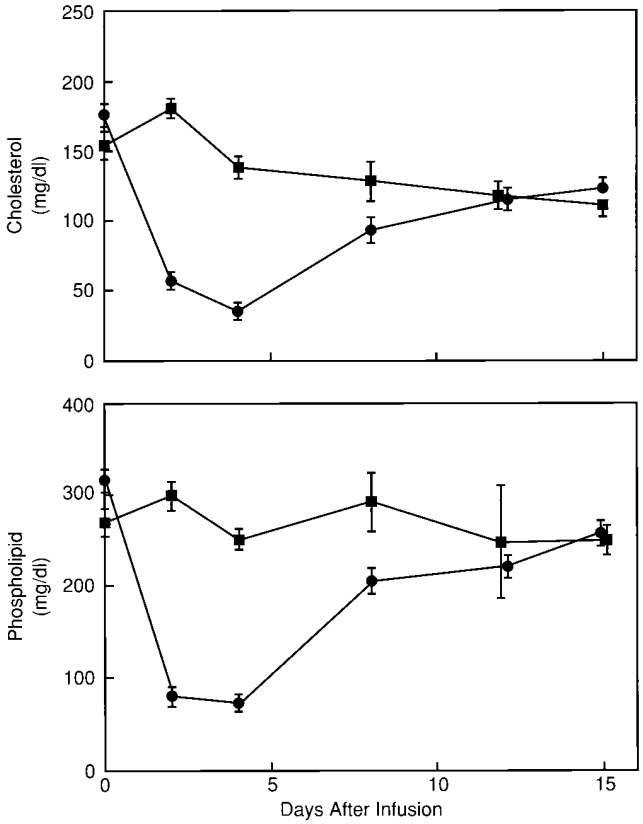

Figure 5. Time course of the cholesterol and phospholipid changes in HL-deficient mice after infusion with HL-rAdV or Lucif-rAdV. The mean data±SEM are shown for the mice treated with HL-rAdV (*circles*) and Lucif-rAdV (*squares*).

Previous studies (10) have demonstrated that in contrast to mouse HL, more than 90% of the heparin-releasable human enzyme is bound to the vascular endothelium in humans. To determine if the expressed human HL would bind to native mouse glycosaminoglycans in a similar manner, we compared the HL activity in pre- and postheparin plasma of HL-deficient mice injected with HL-rAdV with that of control mice. As de-

Table IV. HL Activity in Pre- and Postheparin Plasma of HL-deficient Mice 5 d after Infusion of HL-rAdV or Lucif-rAdV

	HL activity		
	Preheparin	Postheparin*	Postheparin
	nmol/min/ml		%
HL-deficient: day 5			
HL-rAdV ($n = 7$)	918±228	25700±4810	97‡
Lucif-rAdV ($n = 4$)	3±4	8±1	—
Controls ($n = 6$)	130±11	257±32	51

* Postheparin activity contains both pre- and postheparin activities. ‡ $P < 0.005$.

scribed previously in mice (11), 50% of total HL activity was detected in the preheparin plasma of control animals (Table IV), indicating that approximately half of the native mouse enzyme was present in the circulation unbound. In contrast, after infusion with HL-rAdV ~ 97% of the expressed human HL activity was bound and released by heparin (Table IV), demonstrating that structural variability between the two enzymes was responsible for the observed differences in binding to the vascular endothelium.

Discussion

In the past several decades our understanding of the role that different receptors, enzymes, and proteins play in lipid and lipoprotein metabolism has been enhanced greatly. Thus, many of the molecular defects that lead to the human genetic dyslipoproteinemias have been elucidated. In addition, animal models for different genetic disorders have been generated in mice using homologous recombination. These advances now permit the evaluation of different gene therapy approaches for the treatment of human genetic dyslipoproteinemias.

As the major enzyme involved in the hydrolysis of triglycerides and phospholipids present in IDL and HDL, HL plays a central role in lipoprotein metabolism (2, 3). Thus, patients with a deficiency of HL may present with hypercholesterolemia, hypertriglyceridemia, increased plasma HDL concentrations, as well as accumulation of β-VLDL (13–15). Like their human counterparts, HL-deficient mice have increased total plasma cholesterol and HDL concentrations as well as phospholipid enrichment of HDL (23), demonstrating the importance of HL for in vivo HDL remodeling and metabolism in mice.

In this paper we use replication-deficient recombinant adenovirus to replace the human HL gene in HL-deficient mice. One of the major limitations of the currently used adenovirus vector system is the short-term expression of the transgene (40–45) which may be in part related to the development of an immune response directed against viral late gene proteins (46, 47). Despite these limitations, the transient expression achieved using the present generation of recombinant adenovirus permits preliminary assessment of the feasibility of gene replacement in appropriate animal models for ultimate human gene therapy. Thus, recent studies have demonstrated successful transfer of genes coding for circulating plasma proteins (40, 48, 49), cell membrane–associated receptors (39, 41, 50, 51), as well as intracellular enzymes (52) using recombinant adenovirus vectors.

Because of the unique location of HL on the vascular endothelium, HL gene replacement is a more complex process. In addition to delivery of the gene to the liver followed by synthesis, posttranslational modification, and secretion, the mature, active enzyme must ultimately be transported to the capillary endothelium and bind to endothelial glycosaminoglycans for hydrolysis of triglycerides and phospholipids present in circulating plasma lipoproteins (Fig. 2). Thus, we investigated the feasibility of gene therapy for an endothelial-based lipolytic enzyme using replication-deficient adenovirus vectors.

In this report we demonstrate that using this vector system complete correction of the abnormal lipoprotein profile in HL-deficient mice can be achieved. After HL-rAdV infusion, human HL could be detected in mouse postheparin plasma by immunoblotting as well as quantitation of HL lipolytic activity. Expression of the human enzyme was maintained for up to 30 d in treated animals. Fasting plasma cholesterol and phospholipid concentrations were reduced from 176±9 and 314±12 mg/dl to 94±10 and 207±16 mg/dl, respectively, and HDL-C levels were likewise decreased, resulting in normalization of the lipid and lipoprotein profile. The decrease in plasma cholesterol and phospholipid levels due to the increase in HL activity confirms that the expressed HL is active in vivo as a phospholipase (53).

The replacement of the human HL gene in HL-deficient mice has also provided new insights into the previously described differences between the mouse and human enzymes. Thus, in humans most of the HL is heparin releasable (10), indicating that this enzyme is almost entirely bound to glycosaminoglycans in the vascular endothelium. In contrast, a significant amount of mouse HL is found circulating in plasma (11). To date, it is unclear whether these differences in heparin binding are related to variations in the structure of the two enzymes or are determined by differences between the mouse and human endothelial glycosaminoglycans. This question was addressed in the present study by expressing the human enzyme in HL-deficient mice. Our findings demonstrate that despite high levels of expression, more than 95% of the human HL was heparin releasable, indicating binding to the mouse vascular endothelium and establishing that the difference in affinity of the two enzymes for endothelial glycosaminoglycans resides in the structural differences between the mouse and human HL. Thus, the structural variability between the mouse and human HL is responsible for the observed differences in binding to the vascular endothelium.

These combined studies demonstrate successful expression and delivery of a complex lipolytic enzyme to the vascular endothelium using recombinant adenovirus. In addition, adenovirus-mediated replacement of HL has provided new insights into physiologically important structural differences between the mouse and human enzymes, demonstrating a novel application of this vector system for in vivo analyses of lipase structure/function. The successful transport and binding of human HL to mouse endothelial glycosaminoglycans after gene delivery by recombinant adenovirus provides preliminary evidence for replacement of endothelial-bound enzymes in human lipolytic deficiency states for ultimate correction of the gene defects.

Acknowledgments

The authors wish to thank Dr. Frank L. Graham for providing the plasmid vectors pJM17 and pXCX2 used in this study, Dr. Ira J. Goldberg for providing us with the HL goat polyclonal antibody, and Loan Kusterbeck for her assistance in completing the manuscript.

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