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Equivalent binding of wild-type lipoprotein lipase (LPL) and S447X-LPL to GPIHBP1, the endothelial cell LPL transporter

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ABSTRACT

The S447X polymorphism in lipoprotein lipase (LPL), which shortens LPL by two amino acids, is associated with low plasma triglyceride levels and reduced risk for coronary heart disease. S447X carriers have higher LPL levels in the pre- and post-heparin plasma, raising the possibility that the S447X polymorphism leads to higher LPL levels within capillaries. One potential explanation for increased amounts of LPL in capillaries would be more avid binding of S447X-LPL to GPIHBP1 (the protein that binds LPL dimers and shuttles them to the capillary lumen). This explanation seems plausible because sequences within the carboxyl terminus of LPL are known to mediate LPL binding to GPIHBP1. To assess the impact of the S447X polymorphism on LPL binding to GPIHBP1, we compared the ability of internally tagged versions of wild-type LPL (WT-LPL) and S447X-LPL to bind to GPIHBP1 in both cell-based and cell-free binding assays. In the cell-based assay, we compared the binding of WT-LPL and S447X-LPL to GPIHBP1 on the surface of cultured cells. This assay revealed no differences in the binding of WT-LPL and S447X-LPL to GPIHBP1. In the cell-free assay, we compared the binding of internally tagged WT-LPL and S447X-LPL to soluble GPIHBP1 immobilized on agarose beads. Again, no differences in the binding of WT-LPL and S447X-LPL to GPIHBP1 were observed. We conclude that increased binding of S447X-LPL to GPIHBP1 is unlikely to be the explanation for more efficient lipolysis and lower plasma triglyceride levels in S447X carriers.

1. Introduction

Lipoprotein lipase is a triglyceride hydrolase that is responsible for hydrolyzing the triglycerides in triglyceride-rich lipoproteins (TRLs; chylomicrons and VLDL) [1,2]; reviewed in [3]. When LPL is absent, the hydrolysis of plasma triglycerides is severely compromised, leading to markedly elevated plasma triglyceride levels (familial chylomicronemia) [4]. Heterozygosity for LPL deficiency leads to milder forms of hypertriglyceridemia [5,6]. Subtle missense mutations in LPL can impair catalytic activity and lead to small increases in plasma triglyceride levels [7,8]. Elevated plasma triglyceride levels can also be caused by increased production of triglyceride-rich lipoproteins [9,10] or impaired clearance of remnant lipoproteins [11,12]. Of note, LPL can be released from capillaries onto remnant lipoproteins in the plasma. LPL binds to low density lipoprotein receptor family members; hence, LPL could play a role in the clearance of remnant lipoproteins by the liver [13,14].

Approximately 10% of the population carries a single-nucleotide polymorphism in *LPL* (the gene for lipoprotein lipase) that converts Ser-447 to a stop codon, shortening LPL by two amino acid residues [15]. The S447X polymorphism has attracted considerable attention because it lowers plasma triglyceride levels by 10–25% [15] and reduces susceptibility to coronary heart disease [15,16]. However, the mechanism by which this polymorphism affects triglyceride metabolism has remained obscure [15]. One report suggested that the S447X polymorphism alters LPL translation [17], but the mechanism by which this single-nucleotide polymorphism at the end of the coding sequences would alter the efficiency of translation was not clear. Several reports suggested that the S447X polymorphism might increase or decrease LPL activity [18,19], but others have found no significant effect [20–22]. The stabilities of wild-type LPL (WT-LPL) and S447X-LPL in response to denaturation by heat or guanidine hydrochloride are identical [23].

LPL is produced by myocytes and adipocytes but hydrolyzes triglycerides at the luminal face of capillaries. The transport of LPL to the capillary lumen is mediated by GPIHBP1, a GPI-anchored protein of capillary

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endothelial cells [24]. In the setting of GPIHBP1 deficiency, LPL is mislocalized to the interstitial spaces surrounding myocytes and adipocytes and never reaches the capillary lumen, resulting in hypertriglyceridemia [24].

When the amount of LPL in capillaries is negligibly low, as in GPIHBP1 deficiency, the levels of LPL in the pre-heparin plasma are quite low [25,26]. In contrast, S447X carriers have higher-than-normal levels of LPL in the pre-heparin plasma (along with low plasma triglyceride levels) [27], suggesting that the S447X polymorphism leads to higher-than-normal amounts of LPL inside capillaries. GPIHBP1 deficiency also results in lower-than-normal LPL levels in the post-heparin plasma [26,28,29]. In contrast, S447X carriers have significantly higher levels of LPL in the post-heparin plasma [15,30,31]. These contrasting observations have suggested that there might be increased transport of S447X-LPL to the capillary lumen, perhaps due to more avid binding of S447X-LPL to GPIHBP1. The notion that the S447X polymorphism might affect GPIHBP1 binding seemed plausible because LPL binding to GPIHBP1 is mediated by sequences within the carboxyl terminus of LPL [13]. In the current study, we investigated whether the S447X polymorphism affects LPL binding to GPIHBP1. To address this issue, we developed two new binding assays that allowed us to directly compare the abilities of freshly synthesized WT-LPL and S447X-LPL to bind GPIHBP1.

2. Materials and methods

2.1. LPL expression vectors

The S447X polymorphism was introduced into a human LPL expression vector [32] with the QuikChange Lightning kit (Agilent) and oligonucleotide primer 5′-CTGAATAAGAAGTGAGGCTGATCCAC-3′. To create human LPL expression vectors with an internal S-protein (SP) tag, we replaced LPL amino acids 220–233 in the lid domain of LPL [33] with an SP tag (KETAAAKFERQHMDS). The SP tag was amplified from pTriEx-4 (Novagen) with primers 5′-CAGCCAGGATGTAACA TTAAAGAAACCGCTGCTGCG-3′ and 5′-GCACTTCACTAGCTGGTCCGAG TCCATGTGCTGGCG-3′. To create human LPL expression vectors with an internal V5 tag, we replaced LPL amino acids 220–229 in the lid domain of LPL [33] with a V5 tag (GKPIPNPLLGLDST). The V5 tag was amplified from pcDNA3.1 (Life Technologies) with primers 5′–CAGCCAGGATGTAACATTGGTAAGCCTATCCCTAACCC-3′ and 5′-CACTTC ACTAGCTGGTCCACCGTAGAATCGAGACCGAGG-3′. Both tags were introduced into a human LPL expression vector with the InFusion HD cloning kit (Clontech). The constructs encoding the internally tagged LPL proteins were further modified by site-directed mutagenesis to introduce the S447X polymorphism as well as the C418Y mutation into these constructs [13].

2.2. Production of LPL

LPL expression vectors or empty vectors were electroporated into CHO pgsA-745 or CHO-K1 cells with the Nucleofector T kit (5 μg/5 × 10^6 cells). After 2 days, the cell culture medium was collected and concentrated 20-fold with an Amicon Ultra 30,000 MWCO filter. Relative amounts of the internally tagged LPLs (*e.g.,* WT-LPL, S447X-LPL, or C418Y-LPL) in the conditioned medium were assessed by western blots with the LPL-specific antibody 5D2 [34]. Band intensities were quantified with an Odyssey infrared scanner (Li-Cor).

Measurements of WT-LPL or S447X-LPL specific activity were performed by transfecting untagged WT-LPL and S447X-LPL expression vectors into HEK-293 cells (which do not produce LPL). LPL activity in the fresh medium was assessed in quadruplicate with a [^{3}H]triolein substrate (PerkinElmer), and LPL mass was measured with an ELISA with monoclonal antibodies 5D2 and 5F9 [35]. LPL specific activity (nmol/h/μg LPL) was calculated from the activity and mass measurements. We also created immortalized fibroblasts from *Lpl* knockout mice [36], transfected them with the same vectors, and measured LPL specific activity in the same fashion.

2.3. Cell-based LPL–GPIHBP1 binding assay

A GPIHBP1 expression vector was electroporated into CHO-K1 cells (5 μg/5 × 10^6 cells). One day later, GPIHBP1-transfected CHO cells were placed at 4° C for 15 min and washed five times with Dulbecco's phosphate-buffered saline (PBS) containing 100 mg/L $CaCl_2$, 100 mg/L $MgCl_2$, 200 mg/L KCl, 200 mg/ml KH_2PO_4, 8 g/L NaCl, and 2.16 g/L Na_2HPO_4 (Life Technologies). To assess the binding of untagged versions of WT-LPL and S447X-LPL to GPIHBP1, CHO-K1 cells were transfected with either WT-LPL or S447X-LPL plasmids; aliquots of the LPL-containing medium were then added in equivalent and sub-saturating amounts to GPIHBP1-expressing CHO-K1 cells and incubated for 2 h at 4° C [28]. Relative amounts of untagged WT-LPL and S447X-LPL bound to GPIHBP1 were determined by performing western blots of cell extracts [28].

To compare the binding of internally tagged versions of WT-LPL and S447X-LPL to GPIHBP1, equivalent and sub-saturating amounts of the V5- and SP-tagged LPLs (either V5-WT-LPL and SP-S447X-LPL, or SP-WT-LPL and V5-S447X-LPL) were mixed and added to GPIHBP1-expressing cells and incubated for 2 h at 4° C. After washing the cells with PBS, western blots were performed on cell extracts with a mouse V5 monoclonal antibody (Life Technologies) and a goat SP antibody (Abcam) that were labeled with different infrared dyes (IRDye-680 or IRDye-800) (Li-Cor). GPIHBP1 expression was detected with the GPIHBP1-specific antibody 11A12 [28]. Western blot signals were quantified with an Odyssey infrared scanner. The same assay system was used to compare binding of WT-LPL and C418Y-LPL to GPIHBP1. All experiments were performed three times.

2.4. Cell-free LPL–GPIHBP1 binding assay

The binding of internally tagged WT-LPL and S447X-LPL to soluble GPIHBP1 (lacking the GPI anchor) was assessed with a cell-free assay [37,38]. Soluble GPIHBP1 was generated by transfecting CHO pgsA-745 cells with a GPIHBP1 expression vector in which a stop codon was introduced after Gln-197 [28]. After 48 h, the soluble GPIHBP1-containing medium was collected and concentrated 20-fold on an Amicon Ultra 10,000 MWCO filter. The soluble GPIHBP1 was then incubated for 2 h at 4° C with equivalent amounts of internally tagged versions of WT-LPL and S447X-LPL (either V5-WT-LPL and SP-S447X-LPL, or SP-WT-LPL and V5-S447X-LPL) along with agarose beads coated with antibody 11A12. After washing the beads, soluble GPIHBP1 and GPIHBP1-bound LPL were eluted with 0.1 M glycine, pH 2.5. The amounts of GPIHBP1 and LPL in the starting material and in the wash and elution fractions were assessed by western blotting. GPIHBP1 was detected with a GPIHBP1-specific rabbit antibody [32], and the tagged LPLs were detected with V5- and SP-specific antibodies labeled with different IRDyes. Western blot signals were quantified with an Odyssey infrared scanner.

3. Results

Untagged versions of WT-LPL and S447X-LPL were transfected into HEK-293 cells, and fresh medium was collected for LPL mass and activity measurements. The specific activities of WT-LPL and S447X-LPL (measured in quadruplicate) were similar (50.3 ± 0.35 nmol/h/μg for WT-LPL *vs.* 45.4 ± 0.32 nmol/h/μg for S447X-LPL). Similar results were obtained with transfected $Lpl^{-/-}$ fibroblasts, where the specific activity for WT-LPL was ~8% higher than that for S447X-LPL.

To assess the abilities of WT-LPL and S447X-LPL to bind to GPIHBP1, freshly isolated WT-LPL and S447X-LPL preparations were incubated in sub-saturating amounts with GPIHBP1-expressing CHO cells in the presence or absence of heparin. The binding of WT-LPL and S447X-LPL