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Reduced atherosclerosis in chow-fed mice expressing high levels of a catalytically inactive human hepatic lipase

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Abstract

Increased expression of catalytically inactive hepatic lipase (ciHL) lowers remnants and low-density lipoproteins (LDL) and may reduce atherosclerosis in mice lacking both LDLreceptors (LDLR) and murine (m) HL. However, in a previous study, ciHL expression failed to reduce atherosclerosis but increased liver fat accumulation after a 3-month high-fat diet, suggesting that diet-induced metabolic changes compromised the antiatherogenic effects of ciHL. Therefore, we hypothesized that reduced dietary fat would reduce atherosclerosis in ciHL expressing mice. Mice lacking both LDLR and mHL, alone, or expressing ciHL were fed a low-fat (chow) diet for 9 months to match the cumulative cholesterol exposure resulting from a 3-month high-fat diet. Plasma lipids and lipoproteins as well as atherosclerosis were determined at sacrifice. Also, liver expression of receptors and proteins contributing to cholesterol delivery including the LDLreceptor related protein (LRP), scavenger receptor (SR)-B1 and apoE were determined. At 9 months, ciHL expression reduced plasma cholesterol by ∼20% and atherosclerosis by 79% (from 2.67 ± 0.61% of aortic surface, *Ldlr−/−hl−/−*, $n = 9$, to 0.55 ± 0.32% of aortic surface, *Ldlr−/−hl−/−ciHL*, $n = 7$, $P = 0.01$). Also, LRP-expression increased ∼4-fold, whereas SR-B1 and apoE remained unchanged.

These results demonstrate that ciHL expression reduces atherosclerosis. Also, these results demonstrate that ciHL increases LRP expression and suggest increased LRP-mediated lipoprotein clearance as a pathway for ciHL-mediated atherosclerosis reduction.

Keywords: Atherosclerosis; Genetically modified mice; Catalytically inactive hepatic lipase; LRP; Liver lipid accumulation; Heparan sulfate proteoglycans (HSPG)

1. Introduction

Hepatic lipase, a liver produced lipolytic enzyme, plays a critical role in lipoprotein metabolism and influences atherogenesis [1,2]. Hepatic lipase exerts its role using both catalytic and non-catalytic (bridging) functions [3,4]. The catalytic function hydrolyzes triglycerides and phospholipids in remnants, intermediate density lipoproteins (IDL) and high-density lipoproteins (HDL) to form smaller particles for liver uptake (remnants and LDL) or reverse cholesterol transport (HDL) [1,5]. The bridging function facilitates lipoprotein removal through the low-density lipoprotein (LDL) receptor and the LDLreceptor related protein (LRP) [3,6,7], as well as selective cholesteryl-ester uptake through the scavenger receptor (SR)-B1 [8–10]. Thus, HL's catalytic and bridging functions both lower plasma lipoprotein levels, and thereby reduce plasma cholesterol [3,4].

HL's role in atherogenesis is controversial [2,11]. Some studies suggest a proatherogenic role of HL because increased HL activity increases the formation of the atherogenic small dense (sd) LDL [12,13]. Also, increased HL activity reduces plasma levels of the atheroprotective HDL_2 [14]. Thus, despite lowering cholesterol, increased HL activity causes a pro-atherogenic dyslipidemia consisting of sdLDL and low HDL. For example, high HL activity

Abbreviations: HL, Hepatic lipase; LDL, Low density lipoprotein; HDL, High density lipoprotein; LDLR, LDLreceptor; LRP, LDLreceptor related protein; SR-B1, Scavenger receptor B1; ORO, Oil Red O; TBS, Tris buffered saline; HRP, Horse radish peroxidase

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is associated with pro-atherogenic dyslipidemia in the metabolic syndrome, type 2 diabetes mellitus and familial combined hyperlipidemia, all conditions that increase the risk for premature atherosclerosis [15]. Additional evidence for a proatherogenic role is the presence of increased HL levels in men and postmenopausal women at increased risk for atherosclerosis [15]. Evidence for a proatherogenic role for HL is also provided by studies in genetically modified mice. Thus, in apoE deficient mice (an established model of atherosclerosis) the additional absence of mHL by gene targeting reduced atherosclerosis [16]. Interestingly, adding mHL back to macrophages increased atherosclerosis in mice lacking both apoE and mHL [17]. Finally, absence of mHL in mice lacking both apoE and SR-B1 delayed the development of atherosclerosis and prolonged life [18].

Other studies support an antiatherogenic role for HL. For example in humans, HL reduces atherogenic remnants and increases the free cholesterol acceptors HDLsubclass3 (HDL_3) and preβ_1HDL [1]. The HL-facilitated formation of HDL_3 and preβ_1HDL is supported by in vitro studies [19,20]. In mice, expression of HL (reflecting the catalytic function) reduced aortic cholesterol [21]. Also, transgenic overexpression of human HL reduced plasma cholesterol and reduced atherosclerosis [22]. Furthermore, overexpression of a mutant catalytically inactive (ci) HL (reflecting the bridging function), reduced plasma cholesterol suggesting that ciHL has anti-atherogenic properties [3,11]. However, when we tested this possibility in mice fed a high-fat diet, ciHL expression did not reduce cholesterol or atherosclerosis [22]. In fact, ciHL expression was associated with increased liver accumulation of neutral lipids (cholesterol ester and triglyceride), shown by staining of liver sections with Oil Red O. We reasoned that ciHL accelerates liver lipid accumulation by facilitating uptake of lipoproteins that had not been hydrolytically processed in the absence of HL catalytic activity. The accelerated liver lipid accumulation would be caused both by increased particle number and by the unprocessed (non-hydrolyzed) nature of the particles. We postulated that the resultant increase in liver lipid interfered with lipoprotein uptake and cholesterol reduction [22]. This in turn would explain why atherosclerosis was not reduced by ciHL expression in the high-fat fed mice. We reasoned that the considerable reduction in dietary fat content [from 21% (w/w) in the high-fat diet to 4.5% (w/w) in the chow diet] would prevent the accelerated liver lipid accumulation caused by the high fat diet in ciHL expressing mice. Based on the foregoing, we hypothesize that ciHL expression will reduce atherosclerosis in mice fed a low-fat (chow) diet for a period of time calculated to achieve the same cumulative exposure to plasma cholesterol as that achieved with the 3-month high-fat diet. Thus, to test our hypothesis we enrolled nontransgenic mice and mice transgenically expressing high levels of ciHL in a 9-month chow diet study with atherosclerosis development as the study end point.

2. Methods

2.1. Genetically modified mice

Female mice that expressed catalytically inactive human HLS145G (ciHL), (with an inactivating mutation that substitutes a glycine for the catalytic serine at residue 145 [3]) on a background of dual deficiency of the LDLR and mouse HL (*Ldlr−/−hl−/−ciHL*) were generated as described [22]. Specifically, the transgene was designed for liver-specific expression and contains sequences of the human apoE gene including: 3 kilobases (kb) of 5′-flanking sequence, the first exon, the first intron, six nucleotides of the second exon, a polylinker site for insertion of the cDNA, the nontranslated portion of the fourth exon, 0.1 kb of 3′-flanking sequence and the first hepatic control region of the apoE-gene locus [3]. Nontransgenic *Ldlr−/−hl−/−* mice served as controls. Polymerase chain reaction (PCR) analyses of tail DNA confirmed the absence of the genetargeted LDLR and mHL genes and the presence of the ciHL transgene [23,24]. Mice were housed in a full-barrier facility with a 12-h light:12-h dark cycle. All studies were approved by the Institutional Animal Care and Use Committee of the University of Washington.

2.2. Expression of ciHL

The presence of ciHL was confirmed by Western blot analysis of post-heparin plasma using a polyclonal antibody that is specific for human HL [3]. Plasma was collected by retro-orbital bleed 10 min after tail vein injection of heparin (150 U/kg body weight) and stored at −80 °C until use [3]. Also, because expression of the transgene is directed to the liver [3], we examined liver homogenates for ciHL expression by Western blot analysis.

2.3. Lipid and lipoprotein analyses

Cholesterol and triglyceride concentrations were determined on individual fasted plasma from nine *Ldlr−/−hl−/−* and six *Ldlr−/−hl−/−ciHL* mice. For analysis of lipoprotein distribution, fasted plasmas were pooled and fractionated by fast protein liquid chromatography (FPLC) [3]. For *Ldlr−/−hl−/−* mice, FPLC was performed on two pools of two and four individual mice, respectively. For the *Ldlr−/−hl−/−ciHL* mice, FPLC was performed on two pools of three individual mice each. For each genotype the FPLC results were combined into one graphic representation. Standard colorimetric assays were used to measure cholesterol and triglyceride concentrations in plasma and FPLC fractions (cholesterol: Chol 7D62, Abbott Clinical Chemistry, Wiesbaden, Germany; triglycerides: TG kit, Roche/Hitachi, Roche Diagnostics, Mannheim, Germany).