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Overexpression of human ApoAI transgene provides long-term atheroprotection in LDL receptor-deficient mice

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Abstract

The long-term effect of elevated levels of human apolipoprotein AI (apoAI) on atherosclerosis was assessed using human apoAI transgenic mice on a double mutant LDL receptor-deficient (LDLr−/−) and mouse apoAI-deficient (apoAI−/−) background. When they were fed a high fat diet, atherosclerosis in transgenic human apoAI, LDLr−/−, apoAI−/− mice (*hu*apoAITg) was compared with LDLr−/− mice that expressed normal amounts of apoAI (*m*sapoAI) or LDLr−/− mice that lacked mouse apoAI (*no*apoAI). The atheroprotective effect of human apoAI was demonstrated by a greater than six-fold inhibition in lesion areas in the aortic wall and heart valves compared to the two control strains after 27 or 36 weeks. Plasma apoAI concentrations in *hu*apoAITg mice were considerably higher than in *m*sapoAI mice (600 and 37 mg/dL, respectively). The human apoAI transgene led to several plasma HDL subpopulations, with high levels of preβ-HDL and a significant decrease in total plasma cholesterol. This was observed without a change in total HDL cholesterol levels. Thus, elevated levels of human apoAI in LDL receptor-deficient mice lacking mouse apoAI conferred profound protection against diet-induced over extended periods of time.

Keywords: Apolipoprotein AI; HDL; Human apoAI; Transgenic mice

1. Introduction

Low levels of HDL in patients are firmly associated epidemiologically with the risk of coronary heart disease (CHD) [1]. Like other lipoproteins, HDL is a carrier of hydrophobic cholesterol within the aqueous medium of the plasma but differs from the less-dense lipoproteins in its capacity to protect against the deposition of cholesterol within arteries. The size, shape and protein composition of HDL vary significantly depending on cholesterol load and associated enzyme activity. Consistently, however, apoAI plays a central role in the structure and assembly of HDL. This is confirmed by the tight correlation in humans between plasma concentrations of apoAI and HDL cholesterol levels [2]. Patients expressing abnormally low levels of apoAI or in congenital apoAI-deficiency have greater risk of premature CHD [3,4].

A major atheroprotective property of HDL is attributed to its ability to promote cellular cholesterol efflux from peripheral cells to the liver where it can be catabolized [5]. The reverse cholesterol transport (RCT) hypothesis proposes that low HDL levels slow the rate of cholesterol removal from cells such as macrophages that lead to the development of atherosclerotic plaques in arteries. A first step in the RCT pathway is efflux of free cholesterol from cell membranes to lipid poor apoAI in the interstitial fluid via transport by the cellular ATP-binding cassette AI (ABCAI) [6]. These lipid-poor apoAI particles are preβ-migrating lipid-poor complexes of phospholipid and apoAI. After free cholesterol is transferred to these lipid-poor or preβ-HDL particles it is esterified by LCAT to cholesteryl ester which can move into the core of the HDL particle to form α-migrating spherical HDL [7]. In mice, the final step in RCT is the clearance of

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HDL cholesteryl esters predominantly by scavenger receptor class B, type I (SR-BI) in the liver followed by direct or indirect transfer into bile [8,9].

Overexpression of human apoAI reduces atherogenesis, as shown in studies using rabbits fed a hypercholesterolemic diet [10], transgenic C57Bl/6 mice and hyperlipidemic mice [11–13]. A similar effect is observed when apoAI Milano, a natural variant of human apoAI, is infused directly into rabbits [14]. However, the long-term effects of apoAI overexpression have not been examined. Studies using human apoAI transgenic mice that express mouse apoAI have also highlighted differences in the structure, function and metabolism of the apoAI molecule between species [11,15]. Although the HDL particle size profile in human apoAI transgenic mice changes from unimodal to polydisperse similar to that seen in humans, the presence of mouse apoAI might interfere with the HDL complexes through its inability to form large HDL particles by means of its distinct lipid-binding properties [16]. In this study we examined the impact of elevated human apoAI levels on atherosclerosis susceptibility in hypercholesterolemic LDL receptor-deficient (LDLr−/−) mice with special focus on the extended effect of apoAI on lesion progression. In addition, by using a transgenic mouse strain that expressed human apoAI but not mouse apoAI, we examined the physical and anti-atherogenic properties of this human protein without interference from native mouse apoAI.

2. Methods

2.1. Animals and facilities

All mice used in this study were originally obtained from Jackson Laboratories (Bar Harbor) and were on a C57Bl/6 background. ApoAI-deficient mice (apoAI−/−) were crossed with LDLr−/− mice (*m*sapoAI), to generate double-knockout mice (*no*apoAI). Transgenic mice with hepatic overexpression of the human apoAI gene [C57BL/6-TgN(APOA1)1Rub] were crossed with *no*apoAI mice to produce human apoAI transgenic mice that lacked mouse apoAI (*hu*apoAITg). All animals were kept in microisolator cages (12 h light:dark cycle) with free access to autoclaved rodent chow diet (diet no. 5015; Harlan Teklad) and acidified water (pH 2.8). Beginning at 8 weeks of age, 28 males per group were fed a high fat diet (HFD) (diet no. 94059 Harlan Teklad) containing 15.8% (w/w) fat, 1.25% (w/w) cholesterol and no cholate. All procedures were performed in accordance with institutional guidelines.

2.2. Sample collection

Blood was collected using heparinized capillaries from the retro-orbital venous plexus of fasted mice under isoflurane-induced anesthesia and placed in EDTA-coated tubes kept on ice. Plasma was isolated by centrifugation for 5 min at 4 °C and stored at −80 °C until use. Total plasma cholesterol was determined using a commercially available colorimetric kit (Thermo) that was adapted for microplate assays.

2.3. FPLC analysis of lipoprotein distribution

Fractionation of plasma lipoproteins by fast protein liquid chromatography (FPLC) was achieved using two Superdex 200 columns in series on a FPLC system (Pharmacia) as described by Boisvert et al. [17]. Plasma (100 μl) pooled from equal volumes of plasma from five mice per group was chromatographed and 0.5 ml fractions collected. Distribution of cholesterol in the lipoprotein fractions was assayed by a sensitive enzymatic fluorescence method [17]. Levels of human and mouse apoAI were measured by ELISA as described below.

2.4. Preβ-HDL quantitation

Agarose gel electrophoresis of plasma pooled from five mice was performed to determine the distribution of HDL populations with α-and preβ-mobility. Fifteen microliters of pooled plasma collected from mice was loaded onto a 0.75% agarose gel and separated for 2 h at 90 V. The mobility of HDL in plasma from mice that had consumed the chow diet for 8 weeks was compared to that from mice that consumed the HFD for 18 weeks as well as purified mouse apoAI and HDL isolated by ultracentrifugation ($1.063 < d < 1.21$ g/ml). Following semi-dry electrophoretic transfer to a PVDF membrane and blocking overnight at 4 °C with blocking reagents supplied by the manufacturer (Invitrogen), immobilized proteins were incubated with rabbit anti-mouse apoAI (1 μg/ml) (Biodesign International) and rabbit anti-human apoAI (1 μg/ml) (Calbiochem). Rabbit IgG was detected using an HRP-labeled antibody and visualized by chemiluminescence with the ECL kit (Invitrogen).

A similar procedure was followed when separating apoAI-containing lipoproteins by size. A native 4–26% gradient polyacrylamide gel was cast from a 40% acrylamide-bis solution (Bio-Rad) using a dual-chamber gradient maker. Pooled plasma (20 μl) from *m*sapoAI and *hu*apoAITg strains were run at 130 V for 18 h at 4 °C. Following transfer, the separated proteins were visualized using specific antibodies as described above.

2.5. ApoAI determination

Human apoAI was measured in plasma samples and FPLC fractions using a specific sandwich ELISA. MaxiSorp™ treated 96-well flat-bottom microtitre plates (Nunc) were coated overnight at 4 °C with 50 μl of purified rabbit anti-human apoAI polyclonal antibody (1 μg/ml) (Biodesign International). After washing the wells with PBS containing 0.05% Tween and blocking with 10% FBS/PBS for 1 h, diluted samples were added to the wells and incubated for 2 h at RT. The plates were washed and a purified mouse anti-