Atheroprotective Effect of Human Apolipoprotein A5 in a Mouse Model of Mixed Dyslipidemia

Roxane M. Mansouri, Eric Baugeé, Philippe Gervois, Jamila Fruchart-Najib, Catherine Fiévet, Bart Staels, Jean-Charles Fruchart

Hypertriglyceridemia is an independent risk factor for coronary artery disease. Because apolipoprotein (Apo)A5 regulates plasma triglyceride levels, we investigated the impact of human (h)ApoA5 on atherogenesis. The influence of hApoA5 transgenic expression was studied in the ApoE2 knock-in mouse model of mixed dyslipidemia. Our results demonstrate that hApoA5 lowers plasma triglyceride levels in Western diet–fed ApoE2 knock-in mice. Moreover, atherosclerotic lesion development was significantly decreased in the hApoA5 transgenic mice. Finally, pharmacologic activation of hApoA5 expression by the peroxisome proliferator-activated receptor-α agonist fenofibrate resulted in an enhanced atheroprotection. These results identify an atheroprotective role of hApoA5 in a mouse model of mixed dyslipidemia.

ApoA5 is a crucial determinant of plasma triglyceride levels. The apolipoprotein (Apo)A5 gene is located within the ApoA1/C3/A4 gene cluster on human chromosome 11q23. ApoA5 modulates plasma lipid transport. Indeed, ApoA5 deficiency is associated with hypertriglyceridemia both in humans and mice, whereas its overexpression in mice reduces plasma triglyceride levels. Moreover, ApoE2-associated hypertriglyceridemia is ameliorated by adenovirus-mediated ApoA5 overexpression. Because hypertriglyceridemia is an independent risk factor of coronary artery disease (CAD), we investigated the impact of human (h)ApoA5 expression on atherogenesis in human ApoE2 knock-in (ApoE2-KI) mice, which display mixed dyslipidemia and spontaneously develop atherosclerotic plaques. Moreover, because peroxisome proliferator-activated receptor (PPAR)α regulates hApoA5 transcription in vitro, we also assessed the influence of pharmacological modulation of hApoA5 gene expression with the PPARα agonist fenofibrate.

Materials and Methods

Animals and Diets

Homozygous C57BL/6j-ApoE2-KI mice were crossed with C57BL/6j-hApoA5 transgenic mice to obtain ApoE2-KI*hApoA5 mice. At weaning, female mice (n=5 per group) were fed for 8 weeks a chow or Western diet containing (wt/wt) 0.2% cholesterol and 21% fat (UAR, Epinay sur Orge, France). Based on food consumption monitoring, the daily drug delivery of ~100 mg/kg body weight for fenofibrate. Blood was obtained after a 6-hour fasting period by retroorbital puncture under isoflurane-induced anesthesia. This study was conducted according to the Guidelines for the Care and Use of Experimental Animals.

An expanded Materials and Methods section is available in the online data supplement at http://circres.ahajournals.org.

Results

Human ApoA5 Expression Improves Lipid Homeostasis in Chow-Fed ApoE2-KI*hApoA5 Mice

To analyze the impact of hApoA5 on mixed dyslipidemia, hApoA5 transgenic mice were bred with homozygous ApoE2-KI mice to yield ApoE2-KI*hApoA5 mice. Plasma triglycerides were ~2.5-fold lower in chow-fed ApoE2-KI*hApoA5 transgenic versus ApoE2-KI mice (2.01±0.60 versus 4.95±1.50 mmol/L; P<0.001) (Figure 1A) and total cholesterol concentrations were ~2-fold lower (5.59±1.76 versus 12.04±3.47 mmol/L; P<0.005) (Figure 1C). Triglyceride and cholesterol lipoprotein distribution profiles revealed that triglyceride and cholesterol concentrations were strongly decreased in atherogenic particles (very-low-density lipoprotein [VLDL], intermediate-density lipoprotein [IDL], and low-density lipoprotein [LDL]) of ApoE2-KI*hApoA5 transgenic mice compared with ApoE2-KI mice (Figure 1B and 1D). These results demonstrate that hApoA5 improves both triglyceride and cholesterol homeostasis in chow-fed ApoE2-KI*hApoA5 mice.

Human ApoA5 Decreases Triglyceride, but not Cholesterol, Levels in Western-Fed ApoE2-KI*hApoA5 Mice

We next analyzed the impact of hApoA5 on plasma lipid levels in ApoE2-KI*hApoA5 transgenic mice fed a Western diet. Compared with ApoE2-KI mice, hApoA5 mice displayed ~2-fold reduced plasma triglyceride concentrations (2.34±0.64 versus 0.99±0.31 mmol/L; P<0.05) (Figure 1A). Analysis of the lipoprotein fractions revealed that hApoA5 overexpression resulted in a striking decrease of triglycerides in the VLDL, IDL, and LDL fractions (Figure 1B). By contrast, plasma cholesterol levels were not significantly decreased in ApoE2-KI*hApoA5 mice fed the lipid enriched diet (Figure 1C and 1D). Altogether, these results demonstrate that hApoA5 selectively decreases plasma triglycerides in ApoE2-KI*hApoA5 mice fed an atherogenic lipid-enriched diet.

Pharmacological Activation of Human ApoA5 Gene Expression Further Improves Triglyceride Metabolism

We previously demonstrated that hApoA5 is a PPARα target gene in vitro. The nuclear receptor PPARα controls lipid
homeostasis by regulating key genes encoding enzymes and apolipoproteins. PPARα is activated by synthetic fibrate drugs, such as fenofibrate, which are used to normalize hypertriglyceridemia in humans. Interestingly, fenofibrate treatment resulted in a ~2-fold increase of liver hApoA5 mRNA (P<0.05), which was accompanied by elevated plasma protein levels (P<0.05), in ApoE2-KI*hApoA5 mice (Figure 2A). By contrast, murine ApoA5 gene expression was not modified by fenofibrate treatment (data not shown). Analysis of the triglyceride lipoprotein distribution profile revealed that the reduction of atherogenic lipoprotein triglyceride content observed in ApoE2-KI*hApoA5 mice was further enhanced by PPARα activation, resulting in a significant reduction of the VLDL fraction (Figure 2B). By contrast, plasma cholesterol levels were not significantly modified in Western-fed ApoE2-KI*hApoA5 mice treated with fenofibrate (data not shown). Altogether, these data demonstrate that pharmacological activation with fenofibrate induces hApoA5 expression and plasma ApoA5 concentrations in vivo and further improves triglyceride homeostasis.

**Human ApoA5 Decreases Atherosclerosis in Western Diet–Fed ApoE2-KI Mice**

Next, we investigated whether hApoA5 transgenic expression influenced atherogenesis. Atherosclerotic plaque formation was assessed in ApoE2-KI*hApoA5 compared with

Figure 1. Plasma lipids and lipoprotein lipid distribution profiles in ApoE2-KI*hApoA5 vs ApoE2-KI mice. Lipids were measured in ApoE2-KI and ApoE2-KI*hApoA5 mice fed a chow or Western diet. A, Plasma triglyceride levels. B, Lipoprotein distribution profiles of triglycerides. C, Total plasma cholesterol levels. D, Lipoprotein distribution profile of cholesterol. Results are expressed as means±SD (5 mice per group). *P<0.05, **P<0.005, ***P<0.001 vs control group.

Figure 2. Effect of fenofibrate on hApoA5 expression and plasma triglyceride levels in ApoE2-KI*hApoA5 transgenic mice. A, hApoA5 mRNA levels (gray bars) and plasma protein concentrations (black bars) in Western diet–fed ApoE2-KI*hApoA5 mice treated (+) or not (−) with fenofibrate. B, Lipoprotein distribution profile of triglycerides in Western-fed ApoE2-KI*hApoA5 mice treated or not with fenofibrate (FF). Results are expressed as means±SD (5 mice per group). *P<0.05 vs control group.
ApoE2-KI mice by measuring oil red O–stained surfaces at the aortic sinus (Figure 3). Representative photomicrographs showed a decrease of lipid-stained surfaces in aortas of ApoE2-KI (a), ApoE2-KI*hApoA5 (b), ApoE2-KI (c), and ApoE2-KI*hApoA5 (d) mice under Western diet supplemented or not with fenofibrate (FF). Scale bar=500 μm. B, Graphs represent mean lesion area of the analyzed portion. Results are expressed as means±SD (5 mice per group). **P<0.005, ***P<0.001 vs control group.

**Figure 3.** Histological analysis of atherosclerotic lesions. A, Oil red O staining of atherosclerotic lesions in aortas of ApoE2-KI (a), ApoE2-KI*hApoA5 (b), ApoE2-KI (c), and ApoE2-KI*hApoA5 (d) mice under Western diet supplemented or not with fenofibrate (FF). **Discussion**

Hypertriglyceridemia, associated with the metabolic syndrome, is an independent risk factor of CAD, particularly in women. ApoA5 plays a major role in triglyceride homeostasis because human ApoA5 expression reduces hypertriglyceridemia. A recent study demonstrated that adenovirus-mediated ApoA5 expression decreased hypertriglyceridemia in ApoE2-KI mice. It is thought that ApoA5 accelerates hydrolysis of triglyceride-rich lipoproteins by proteoglycan bound lipoprotein lipase.

Here, we addressed the impact of human ApoA5 transgenic expression on atherogenesis in vivo, by generating hApoA5 transgenic mice in the ApoE2-KI background. Our results confirm that permanent transgenic expression of hApoA5 results in a decrease of plasma triglyceride levels in chow-fed mice. Moreover, we observed that hApoA5 lowered plasma cholesterol levels in chow-fed ApoE2-KI mice. Interestingly, also in Western diet-fed ApoE2-KI*hApoA5 mice, hApoA5 decreased plasma triglyceride levels. This improvement of lipid homeostasis was related to a decrease of triglyceride content in proatherogenic particles. By contrast, plasma cholesterol levels were not modified in ApoE2-KI*hApoA5 mice on a Western diet. Previous studies have demonstrated that elevated serum ApoA5 levels are associated with a decrease in serum triglycerides and cholesterol in ApoE2-KI mice fed a chow diet. Thus, serum level of ApoA5 reached in our transgenic model was probably not sufficiently high to correct the hypercholesterolemia in ApoE2-KI mice fed a Western diet.

Strikingly, the decrease of plasma triglyceride levels in the ApoE2-KI*hApoA5 on the Western diet was associated with an ~2-fold decrease of atherogenesis. Our findings thus identify ApoA5 as an antiatherogenic factor, which may, at least in part, act through an improvement of triglyceride homeostasis. These effects, however, do not exclude alternative or complementary mechanisms of action of hApoA5 in atheroprotection.

We previously reported that hApoA5 is a PPARα target gene in vitro, whereas murine ApoA5 expression is not altered by the pharmacological activation of PPARα. Indeed, a PPARα response element was identified in the human promoter region of the ApoA5 gene. However, sequence analysis of the murine ApoA5 promoter did not reveal a putative peroxisome proliferator response element and functional analyzes by transfection and EMSA experiments demonstrated that PPARα activation does not alter murine ApoA5 gene expression. The present work extends these observations to the in vivo situation. Accordingly, a recent pharmacogenetic study implicated genetic variation in hApoA5 as a determinant of the plasma lipoprotein response to fibrates. The upregulation of hApoA5 by PPARα may contribute to its effect on triglyceride homeostasis. ApoA5 is present at very low plasma concentrations. However, ApoA5 is a major regulator of triglyceride metabolism in humans. Therefore, it is conceivable to assume that a small but significant raise of hApoA5 expression on PPARα activation may participate in the strong decrease of plasma triglyceride levels in ApoE2-KI*hApoA5 mice treated with fenofibrate.

Because of the atherogenic potential of hypertriglyceridemia, the use of strategies to manage triglyceride levels is warranted to further reduce excessive residual CAD risk. Fenofibrate has been shown to reduce plasma triglyceride levels. The fact that hApoA5 prevents atherosclerotic lesion formation in a mouse model of mixed dyslipidemia, an effect likely attributable to the lowering of plasma triglyceride levels, suggests that the increase of hApoA5 plasma
levels after fenofibrate may contribute to confer maximal atheroprotection.

In conclusion, even though the molecular mechanisms by which ApoA5 decreases plasma triglycerides remain to be firmly established, our observations provide evidence that, in vivo, human ApoA5 displays antiatherogenic properties. These results strongly reinforce the interest in human ApoA5 as a target for the treatment of hypertriglyceridemia and atherogenesis.

Acknowledgments
We thank E. Vallez for technical assistance, R. Bordet for loaning material, and J. Dallongeville for statistical analyses.

Sources of Funding
This work was supported by grants from Agence Nationale de la Recherche and Genfit SA (COMAX), Région Nord-Pas de Calais/ FEDER, Fondation Coeur et Artères, and the European Vascular Genomics Network.

Disclosures
None.

References

KEY WORDS: human apolipoprotein A5 | atherosclerosis | dyslipidemia | fibrates | mouse model
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Circ Res. 2008;103:450-453; originally published online July 24, 2008;
doi: 10.1161/CIRCRESAHA.108.179861

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/103/5/450

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2008/07/24/CIRCRESAHA.108.179861.DC1
**Supplementary Materials and Methods**

**RNA extraction and quantitative PCR analysis**

RNA was isolated from the liver using the acid guanidium thiocyanate/phenol/chloroform method\(^1\). Reverse transcription was realized on 1μg RNA using random hexameric primers and Superscript reverse transcriptase (Invitrogen). Human ApoA5 mRNA was measured by real-time quantitative PCR using Brilliant SYBR Green Q-PCR Master Mix (Stratagene) on the Mx4000 detection system (Stratagene) using the specific primers 5’-CAGGGTCGAGGGCTCTTGTCCT-3’ and 5’-CCCGCTGCACTCTCCAGAAT-3’.

**Biochemical analyses**

At the end of the study, blood samples were drawn. Plasma was then separated for analyses. Plasma cholesterol and triglyceride levels were measured using commercially available kits\(^2\). Lipoprotein triglyceride and cholesterol distribution profiles were analyzed in pooled plasma samples from each group by gel filtration chromatography using a Superose 6 HR 10/30 column (Amersham Pharmacia Biotech). Triglyceride and cholesterol concentrations were determined in the eluted fractions.

**Analysis of atherosclerotic lesions**

Mice were euthanized by cervical dislocation, the heart of each animal was fixed with 4% phosphate-buffered paraformaldehyde (pH 7.0) and serial 10-μm-thick sections were cut between the valves and the aortic arch for quantitative analysis of lipid deposition by Oil red-O\(^3\). Each section was recorded using a Nikon microscope (Diaphot; Nikon France S.A, Champigny sur Marne, France) and a color video camera (Sony CCD IRIS DXC 107 AP; SONY France, Paris, France). Color images were acquired using a PC fitted with a frame-
grabbing board (Snappy, Video Snapshot; HCS MISCO, Verrières le Buisson, France). Quantification of atherosclerotic lesion area was performed using Scion Image software.

**Statistical Analysis**

Significant differences between means were determined by ANOVA comparison followed by a post-hoc analysis. The SPSS software release 7.5 for windows was used (SPSS Institute Inc., Paris, France). A value of $P<0.05$ was considered as statistically significant.

**References**

