Associations of ApoAI and ApoB-containing Lipoproteins with AngII-induced Abdominal Aortic Aneurysms in Mice

Jing Liu[1,2*], Hong Lu[1*], Deborah A. Howatt[1], Anju Balakrishnan[1],

Jessica J. Moorleghen[1], Mary Sorci-Thomas[3], Lisa A. Cassis[2] and Alan Daugherty[1,2]

[1]Saha Cardiovascular Research Center
[2]Department of Pharmacology and Nutritional Sciences
University of Kentucky, Lexington, KY

[3]Department of Medicine
Medical College of Wisconsin, Milwaukee, WI

* These authors contributed equally

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Materials and Methods

Mice and Diets

C57BL/6J, apoA-I -/-, LDL receptor -/-, and apoE -/- mice were purchased from The Jackson Laboratory (Stock # 0664, 2055, 2207, and 2052, respectively; Bar Harbor, ME, USA). Breeding pairs of apoA-I +/+ x LDL receptor -/- and apoA-I -/- x LDL receptor -/- mice were developed by Dr. Sorci-Thomas.[1] Mice were maintained in individually vented cages (maximally 5 mice/cage) on a light:dark cycle of 14:10 hours. The cage bedding was Teklad Sani-Chip bedding (Cat # 7090A, Harlan Teklad, Madison, WI, USA). Mice were fed a normal rodent laboratory diet (Diet # 2918, Harlan Teklad; Madison, WI, USA) and given drinking water from a reverse osmosis system ad libitum. During experiments, mice were either continuously fed the normal diet or a Western diet (Diet # TD.88137; Harlan Teklad) containing 21% (wt/wt, which equals 42% calories/calories) saturated fat extracted from milk, 48.5% (wt/wt) carbohydrate, 17.3% (wt/wt) protein, and 0.2% (wt/wt) cholesterol (0.15% supplemented, and 0.05% from the fat source) 1 week prior to and during 4 weeks of AngII infusion. Ezetimibe was provided by Merck & Company, Inc. Diets containing ezetimibe (50 mg/kg) were customized by Harlan Teklad. All procedures were performed with the approval of the University of Kentucky Institutional Animal Care and Use Committee.

Osmotic Mini Pump Implantation and AngII Infusion

AngII (1,000 ng/kg/min or 500 ng/kg/min; Cat# H-1706; Bachem; Torrance, CA, USA) was infused subcutaneously via Alzet osmotic mini pumps (Alzet Model # 2004; Durect; Cupertino, CA, USA) as described previously.[2] Only apoAI +/+ and -/- mice in an LDL receptor -/- background fed Western diet were infused with AngII 500 ng/kg/min. Mice were sedated with isoflurane and pumps were implanted subcutaneously on the right flank of each mouse. Surgical staples were used to close the incision site and a topical anesthetic cream (LMX4; Ferndale Laboratories; Ferndale, MI) was applied immediately after surgery to relieve pain.

Systolic Blood Pressure Measurements

Systolic blood pressure was measured using a standardized protocol described previously[3] on conscious mice for 3 consecutive days prior to (baseline) and during the last week of AngII infusion by a non-invasive tail-cuff system (Coda 8; Kent Scientific; Torrington, CT, USA).

Plasma Measurements

Mice were anesthetized using ketamine/xylazine cocktail at termination. Blood samples were harvested by right ventricular puncture with EDTA (final concentration: 1.8 mg/ml) and then centrifuged at 400 g x 20 minutes, 4 °C to prepare plasma.

Plasma cholesterol concentrations were measured using an enzymatic kit (Cat # 439-17501; Wako Chemicals USA, Richmond, VA, USA). Plasma lipoprotein distributions were resolved by size exclusion gel chromatography followed by enzymatic measurement of cholesterol in each collected fraction, as described previously.[4]

Quantification of Aortic Pathologies

At termination, after blood collection, right atrium was cut open, and saline was perfused through the left ventricle to remove blood from the systemic circulation. Subsequently, aortas were dissected and placed in 10% neutrally buffered formalin overnight at room temperature. After fixation, periaortic adventitia was carefully removed.

Necropsies were performed for mice died during AngII infusion. Aortic rupture was defined as observation of blood clots in either the thoracic cavity (thoracic aortic rupture) or retroperitoneal cavity (abdominal aortic rupture). Maximal outer diameter of the suprarenal aorta was measured ex vivo as a parameter for abdominal aortic aneurysm (AAA) quantification using Image-Pro software (Version 7; Media Cybernetics; Bethesda, MD, USA). Thoracic aortas were cut open and pinned for quantification of intimal area and atherosclerotic lesion area in a region including ascending, arch and 3 mm of descending aorta using an en face technique.[5,6] Atherosclerosis was compared between groups with percent lesion area (lesion area/intimal area x 100%).

Statistical Analyses

Data were analyzed using SigmaPlot version 12 (SYSTAT Software Inc., Chicago, IL, USA). Data are represented as means ± standard error of means (SEM). To compare two study groups of each experiment on a continuous variable, unpaired two-tailed Student's t test was performed for normally distributed and equally variant values, and Mann-Whitney rank sum test were used for non-normally distributed variables. Systolic blood pressure were analyzed using two-way repeated measures ANOVA. $P < 0.05$ was considered statistically significant.

References

1. Zabalawi M, Bhat S, Loughlin T, Thomas MJ, Alexander E, Cline M, Bullock B, Willingham M, SorciThomas MG. Induction of fatal inflammation in LDL receptor and ApoA-I double-knockout mice fed dietary fat and cholesterol. *American Journal of Pathology* 2003;163:1201-1213.

2. Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. *J Clin Invest* 2000;105:1605-1612.

3. Daugherty A, Rateri D, Lu H, Balakrishnan A. Measuring blood pressure in mice using volume pressure recording, a tail-cuff method. *J Vis Exp* 2009;27:1291.

4. Daugherty A, Rateri DL. Presence of LDL receptor-related protein/alpha 2-macroglobulin receptors in macrophages of atherosclerotic lesions from cholesterol- fed New Zealand and heterozygous Watanabe heritable hyperlipidemic rabbits. *Arterioscler Thromb* 1994;14:2017-2024.

5. Daugherty A, Whitman SC. Quantification of atherosclerosis in mice. *Methods Mol Biol* 2003;209:293-309.

6. Daugherty A, Rateri DL. Development of experimental designs for atherosclerosis studies in mice. *Methods* 2005;36:129-138.