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Increased atherosclerosis in mice with vascular ABCG1 deficiency

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Abstract

Objective—The objective of this study was to investigate the role of vascular ABCG1 in atherogenesis without a confounding difference in macrophage ABCG1 expression. The ATP Binding Cassette Transporter G1 (ABCG1) is highly expressed in macrophages and endothelial cells. ABCG1 preserves endothelial function by maintaining endothelial nitric oxide synthase (eNOS) activity and by reducing adhesion molecule expression and monocyte adhesion.

Methods and Results—To investigate the role of vascular ABCG1 expression in atherosclerosis *in vivo*, *Abcg1*$^{-/-}$*Ldlr*$^{-/-}$ and *Ldlr*$^{-/-}$ mice were transplanted with wild-type bone marrow and fed a Western type diet for 12 or 23 weeks. The atherosclerotic lesion area was similar in both groups after 12 weeks, but was increased in *Abcg1*$^{-/-}$*Ldlr*$^{-/-}$ recipients after 23 weeks, especially in the aortic arch (2.2-fold; $P<0.01$). ENOS-mediated vascular relaxation was impaired in male *Abcg1*$^{-/-}$*Ldlr*$^{-/-}$ recipients.

Conclusion—Our data show an athero-protective role of vascular ABCG1 especially in the aortic arch, likely related to its role in preservation of eNOS activity.

Keywords

ABCG1; HDL; atherosclerosis; eNOS; endothelium

The cholesterol efflux promoting ATP-Binding Cassette Transporter G1 (ABCG1) is highly expressed in macrophages and endothelial cells (ECs)1,2 where it helps to preserve eNOS activity1 and likely reduces expression of vascular adhesion molecules, chemokines, and monocyte adhesion.3 Large high density lipoproteins (HDLs) that result from treatment with niacin or cholesteryl ester transfer protein inhibitors efficiently promote cholesterol efflux

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from ABCG1-expressing cells,4 suggesting that endothelial ABCG1 might promote beneficial effects of HDL raising therapies on vascular functions.

Reduced eNOS activity and increased expression of adhesion molecules are features of atherosclerotic lesions in humans.5 ENOS deficiency accelerates atherogenesis in apolipoprotein E deficient (*Apoe*$^{-/-}$) mice, whereas hypomorphic vascular cell adhesion molecule-1 (VCAM-1) mice show decreased atherogenesis.6,7 These studies suggest the hypothesis that vascular ABCG1 expression is anti-atherogenic. To investigate the role of vascular ABCG1 in atherosclerosis *in vivo*, we transplanted *Abcg1*$^{-/-}$*Ldlr*$^{-/-}$ and *Ldlr*$^{-/-}$ mice with wild-type bone marrow (BM).

Methods

Ldlr$^{-/-}$ and *Abcg1*$^{-/-}$*Ldlr*$^{-/-}$ mice were transplanted with wild-type BM and fed a Western type diet (WTD) for 12 or 23 weeks. Atherosclerotic lesion area was assessed. The supplementary data contain additional information (available online at http://www.atvb.ahajournals.org).

Results

To investigate the role of vascular ABCG1 in atherogenesis, we transplanted *Ldlr*$^{-/-}$ and *Abcg1*$^{-/-}$*Ldlr*$^{-/-}$ mice with wild-type BM. The efficiency of BM reconstitution was greater than 90%. Five weeks after transplantation, animals were fed a WTD for 12 weeks. Cholesterol levels did not differ between the two groups (Supplemental Table I). The atherosclerotic lesion area in the aortic root was similar in the 2 groups; there was minimal lesion formation and no difference in the aortic arch and descending aorta (Supplemental Figure IA and B). However, after feeding the WTD for 23 weeks, *Abcg1*$^{-/-}$*Ldlr*$^{-/-}$ recipients showed a 20% increase in atherosclerosis in the aortic root (Figure 1A, Supplemental Figure II) ($P<0.05$), a 2.2-fold increase in the aortic arch ($P<0.01$) (Figure 1B and D), and no difference in the descending aorta (Figure 1C and D). Combined data from the aortic arch and descending aorta showed a 1.9-fold increase in lesions in *Abcg1*$^{-/-}$*Ldlr*$^{-/-}$ recipients ($P<0.05$) (Supplemental Figure IIIA). The difference was significant in males and females (2.2-fold and 1.6-fold, respectively; $P<0.05$ for both) (Supplemental Figure IIIB and C).

To investigate vascular function in *Abcg1*$^{-/-}$*Ldlr*$^{-/-}$ and *Ldlr*$^{-/-}$ recipients a WTD, femoral arteries were preconstricted with phenylephrine, and responses to endothelium-dependent acetylcholine (ACh) and smooth muscle cell (SMC)-dependent sodium nitroprusside (SNP) vasodilating agents were measured. Male *Abcg1*$^{-/-}$*Ldlr*$^{-/-}$ recipients showed reduced vasorelaxation in response to ACh (Figure 2A), reflected by an increased EC_{50} value compared to *Ldlr*$^{-/-}$ recipients (EC_{50}, 57.3±14.8 nM versus 15.4±5.0 nM) ($P<0.05$). ACh-induced vasorelaxation was similar in female *Abcg1*$^{-/-}$*Ldlr*$^{-/-}$ and *Ldlr*$^{-/-}$ recipients (Figure 2B) (EC_{50}, 29.2±8.0 nM versus 25.5±13.3 nM). SNP-induced vasorelaxation was not affected by the recipient genotype (Figure 2C and D). We also analyzed VCAM-1 expression in the EC layer of the aortic root. VCAM-1 expression tended to increase in males but not females (Supplemental Figure IVA–C), but data did not reach significance. Aortic arch samples were not available for analysis.

Discussion

The role of ABCG1 in atherogenesis is complex, but important in view of its potential role in response to HDL raising therapies. Although *Abcg1*$^{-/-}$ mice show prominent foam cell accumulation in tissues,2 most, but not all, reports have found unchanged or reduced

atherosclerosis in susceptible mice transplanted with *Abcg1*$^{-/-}$ BM.[8,9,10] Studies in *Abcg1*$^{-/-}$ mice have also led to conflicting reports.[11,12] In *Abcg1*$^{-/-}$*Apoe*$^{-/-}$ mice fed WTD, atherosclerosis was decreased,[12] whereas in *Abcg1*$^{-/-}$ mice with small foam cell lesions fed the Paigen diet atherosclerosis was increased.[11] The present study provides the first demonstration of an athero-protective role for vascular ABCG1. Because ABCG1 is highly expressed in ECs and undetectable in vascular SMCs,[1,2] our results are most likely caused by endothelial ABCG1 expression, and are consistent with earlier studies indicating an important role of ABCG1 in preserving endothelial functions.[1,3]

Interestingly, the increase in atherosclerosis in *Abcg1*$^{-/-}$*Ldlr*$^{-/-}$ recipient mice after 23 weeks of diet was most pronounced in the aortic arch. Increased atherosclerosis in *Enos*$^{-/-}$*Apoe*$^{-/-}$ mice is also most prominent in this location.[7] The aortic arch is exposed to altered blood flow, reducing eNOS activity and NO bioavailability.[13] We found decreased endothelium-dependent vasorelaxation reflecting decreased eNOS activity and NO production in *Abcg1*$^{-/-}$*Ldlr*$^{-/-}$ male but not female recipients. Although males clearly had increased atherosclerosis in the arch, our samples size was too small to test for gender-specific differences. Sex differences in eNOS activity have been attributed to eNOS activity-inducing estrogens in females.[14] NO has several anti-atherogenic properties, including attenuation of EC-leukocyte interactions.[15] *Abcg1*$^{-/-}$ ECs also show increased expression of VCAM-1 and E-selectin.[3] A variety of mechanisms are likely responsible for the atheroprotective functions of vascular ABCG1. Evaluations of VCAM-1 expression in the present study were limited, and further investigations are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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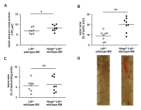

Figure 1.

Atherosclerosis in *Ldlr*$^{-/-}$ (n=8) and *Abcg1*$^{-/-}$*Ldlr*$^{-/-}$ mice (n=9) transplanted with wild-type BM (23 weeks WTD). Haematoxylin-eosin staining was performed on paraffin sections of the aortic root and Oil Red O staining on the aortic arch and descending aorta. Atherosclerotic lesion area in (**A**) the aortic root, (**B**) the aortic arch, and (**C**) the descending aorta. **D**. Representative pictures of Oil Red O staining in the aortic arch and descending aorta. In A–C, each point represents an individual mouse. **P*<0.05, ***P*<0.01.

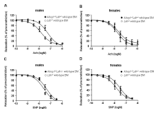

Figure 2.

Response to vasodilating agents in femoral arteries of $Ldlr^{-/-}$ (n=4 per sex) and $Abcg1^{-/-}Ldlr^{-/-}$ (n=4 per sex) mice transplanted with wild-type BM (12 weeks WTD). Response to acetylcholine (Ach) (**A–B**) and sodium nitroprusside (SNP) (**C–D**).