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EDITORIAL

A New Small Molecule Increases Cholesterol Efflux

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Cholesterol efflux is the first step in reverse cholesterol transport, the removal of cholesterol from macrophage foam cells in the arterial wall by HDLs (high-density-lipoproteins), transport in plasma, uptake by the liver and ultimate secretion into the bile.[1] While the cholesterol transporter ABCA1 (ATP-binding cassette A1) mediates cholesterol efflux to apolipoprotein A1 and small HDL particles, ABCG1 mediates cholesterol efflux to mature HDL.[2–4] Studies in animal models have shown antiatherogenic roles for Abca1- and Abcg1-mediated cholesterol efflux pathways in macrophages, hematopoietic stem and progenitor cells, and endothelial cells.[5–9] Studies in large population cohorts have shown that the cholesterol efflux capacity of HDL (ie, its potential to act as an acceptor for cholesterol efflux from macrophages) is an inverse predictor of cardiovascular disease.[10–12] Hence, enhancing cholesterol efflux is a highly desirable therapeutic approach to decrease cardiovascular risk. While agonists of the transcription factor the liver X receptor (LXR) that upregulates Abca1 and Abcg1 expression[2,3,13] have been developed for this purpose,[14,15] their therapeutic benefit has been compromised by adverse effects such as hepatic steatosis and elevated plasma LDL (low-density-lipoprotein)-cholesterol levels.[15–17]

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In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Xu et al[18] identify the small molecule E17241 in an ABCA1 promoter luciferase reporter assay high-throughput screen in HepG2 cells. E17241 dose-dependently upregulates *Abca1* mRNA expression in macrophages and hepatocytes and increases macrophage cholesterol efflux to apolipoprotein A-I in vitro and macrophage reverse cholesterol transport in vivo. At 2 different doses, daily gavage of E17241 showed atheroprotective effects in mice deficient in apolipoprotein E (*Apoe*$^{-/-}$ mice) fed a Western-type diet, accompanied by increases in Abca1 protein expression in macrophages of atherosclerotic plaques and in hepatocytes.[18] Plasma levels of HDL-cholesterol were not affected, presumably because hepatic levels of the scavenger receptor BI (SR-BI) were also increased by E17241. E17241 decreased plasma ALT (alanine aminotransferase) and AST (aspartate transaminase) levels, suggesting no hepatic toxicity.[18] Hence, E17241 upregulates Abca1 in macrophages and hepatocytes without adverse effects on the liver.

E17241 enhances the activity of PKCζ (protein kinase C ζ),[18] which phosphorylates the specificity protein 1 (Sp1) element in the Abca1 promoter, and induces Abca1 expression in macrophages.[19] Upon phosphorylation, Sp1 helps recruitment of the LXR/retinoid X receptor heterodimer to the Abca1 promoter by physical binding.[19,20] Indeed, the effect of E17241 was dependent on the direct repeat of 2 hexameric binding motifs spaced by 4 nucleotides (DR4) element in the Abca1 promoter, where the LXR/retinoid X receptor heterodimer binds (Figure).[18] E17241, by inducing PKCζ activity, enhanced expression of the transcription factors LXRα, LXRβ, retinoid X receptor, PPAR (peroxisome proliferator-activated receptor)α, PPARγ, and PPARδ in luciferase assays in HepG2 cells, suggesting a broad spectrum of action.[18] However, these data were obtained in an overexpressor system and need to be confirmed in vivo. E17241 enhanced hepatic SR-BI expression, which was proposed to be mediated by LXR activation. While this requires mechanistic evidence, livers from mice treated with E17241 showed a decrease in triglyceride accumulation, suggesting that other LXR target genes, such as those that enhance lipogenic gene expression, were not affected by E17241. Hence, E17241 does not activate all LXR target genes in hepatocytes, and perhaps increases SR-BI

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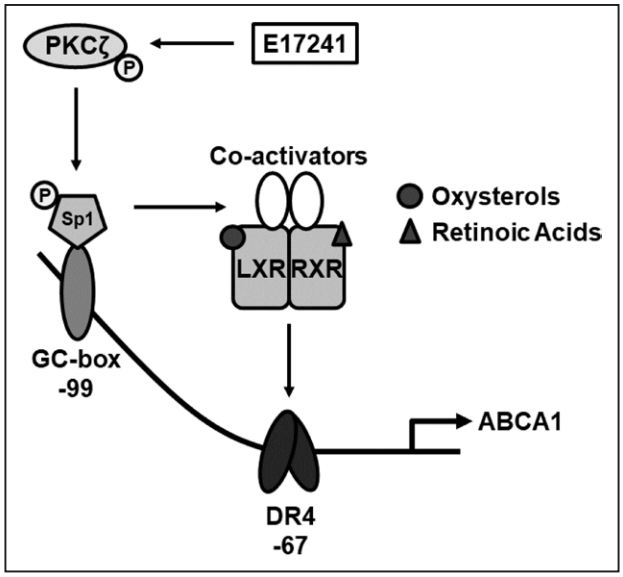

Figure. Proposed model of E17241 mediated effects on regulatory elements of the ABCA1 (ATP-binding cassette A1) gene promoter.
The small molecule E17241 phosphorylates PKCζ (protein kinase C ζ), leading to its activation. Activated PKCζ phosphorylates Sp1 (specificity protein 1), which binds to the ABCA1 promoter. When the liver X receptor (LXR) and retinoid X receptor (RXR) are activated by their ligands, the LXR/RXR heterodimer is formed. Sp1 helps recruitment of the LXR/RXR heterodimer to the DR4 regulatory element on the ABCA1 promoter, presumably by forming a multiprotein complex with the heterodimer and co-activators. This upregulates ABCA1 gene expression.

expression via an indirect LXR-mediated mechanism, similar to its effects on ABCA1. The mechanisms for the decrease in hepatic TG warrant further investigation.

Xu et al extended their findings on E17241 in mice to golden hamsters fed Western-type diet. E17241 did not elicit hepatic toxicity in hamsters. While E17241 tended to increase fecal cholesterol in *Apoe*$^{-/-}$ mice, these effects reached statistical significance in hamsters and may be the result of increased reverse cholesterol transport or direct effects of E17241 on fecal cholesterol excretion. Unlike in Western-type diet-fed *Apoe*$^{-/-}$ mice, E17241 decreased plasma LDL-cholesterol levels and plasma TG levels in hamsters.[18] ApoE and CETP (cholesteryl ester transfer protein) expression in hamsters may contribute to these beneficial effects. Hence, E12741 may affect the expression of more genes than Abca1 alone. While E17241 increased the expression of Abca1 in hepatocytes and macrophages, it lowered plasma glucose levels.[18] Hepatic Abca1 expression decreases glucose levels; however, this effect is downstream of increased plasma HDL.[21] Plasma HDL was not affected by E17241.[18] E17241 may reduce glucose levels by increasing Abca1 expression in β-cells directly.[22] Effects of E17241 on Abca1 expression in other tissues, as well as mechanisms for its TG- and LDL-lowering effects would need to be elucidated.

Abca1 expression is anti-inflammatory.[23–26] Macrophage *Abca1* deficiency increases toll-like receptor 4 and MyD88 signaling,[23–25] and combined deficiency of *Abca1* and *Abcg1* in macrophages enhances proinflammatory gene expression in atherosclerotic plaques.[6] Heterozygous *ABCA1* mutation carriers show increased systemic and vascular inflammation.[27] Moreover, patients with Tangier Disease with homozygous *ABCA1* loss-of-function mutations show inflammasome activation.[26] Therefore, E17241 could suppress inflammation by upregulating Abca1 expression, contributing to its antiatherogenic effects.

The question remains as to how E17241 compares to known compounds that upregulate Abca1 expression, such as antagomirs to microRNA(miR)-33, and LXR agonists. MiR-33 antagomirs and LXR agonists have a wide range of effects. To circumvent the adverse effects of LXR agonism on hepatic steatosis and increasing plasma LDL-cholesterol levels downstream of the LXR target gene the inducible degrader of the LDL receptor (IDOL) in the liver,[15–17] nanoparticles targeted to the vessel wall encapsulating the LXR agonist GW3965, have been developed.[28] These nanoparticles decrease atherosclerosis in *Ldlr*$^{-/-}$ mice and upregulate *Abca1* mRNA expression in CD68^{+} macrophages of the atherosclerotic plaque, while downregulating inflammatory gene expression, and not affecting hepatic LXR target genes.[28] These data suggest that nanoparticles containing GW3965 have therapeutic potential for cardiovascular disease. E17241 has the advantage over a nanoparticle that it exerts its antiatherogenic effects upon oral administration.

MiR-33 antagomirs decrease atherosclerosis progression and induce atherosclerosis regression in mice by increasing Abca1 expression in macrophages and hepatocytes[29,30] and also increase HDL and decrease VLDL-TG in nonhuman primates.[31] While an elegant study has shown that the antiatherogenic effects of miR-33 deficiency were dependent on macrophage Abca1 expression,[32] miR-33 has a multitude of target genes. Whole-body miR-33 deficiency induces food intake, obesity, and insulin resistance.[33] Hence, similar to LXR agonists, miR-33 antagomirs may require tissue-specific targeting to exert antiatherogenic effects.

In conclusion, Xu et al identified a new small molecule that upregulates Abca1 expression in macrophages and hepatocytes. If E17241 is indeed highly specific for Abca1, it may hold great promise as a lead compound for cardiovascular therapy.

ARTICLE INFORMATION

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Disclosures

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REFERENCES

1. Glomset JA. The plasma lecithins: cholesterol acyltransferase reaction. *J Lipid Res*. 1968;9:155–167.
2. Kennedy MA, Barrera GC, Nakamura K, Baldán A, Tarr P, Fishbein MC, Frank J, Francone OL, Edwards PA. ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. *Cell Metab*. 2005;1:121–131. doi: 10.1016/j.cmet.2005.01.002
3. Wang N, Lan D, Chen W, Matsuura F, Tall AR. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. *Proc Natl Acad Sci USA*. 2004;101:9774–9779. doi: 10.1073/pnas.0403506101
4. Wang N, Silver DL, Costet P, Tall AR. Specific binding of ApoA-I, enhanced cholesterol efflux, and altered plasma membrane morphology in cells expressing ABC1. *J Biol Chem*. 2000;275:33053–33058. doi: 10.1074/jbc.M005438200
5. Out R, Hoekstra M, Habets K, Meurs I, de Waard V, Hildebrand RB, Wang Y, Chimini G, Kuiper J, Van Berkel TJ, et al. Combined deletion of macrophage ABCA1 and ABCG1 leads to massive lipid accumulation in tissue macrophages and distinct atherosclerosis at relatively low plasma cholesterol levels. *Arterioscler Thromb Vasc Biol*. 2008;28:258–264. doi: 10.1161/ATVBAHA.107.156935
6. Westerterp M, Murphy AJ, Wang M, Pagler TA, Vengrenyuk Y, Kappus MS, Gorman DJ, Nagareddy PR, Zhu X, Abramowicz S, et al. Deficiency of ATP-binding cassette transporters A1 and G1 in macrophages increases inflammation and accelerates atherosclerosis in mice. *Circ Res*. 2013;112:1456–1465. doi: 10.1161/CIRCRESAHA.113.301086
7. Westerterp M, Tsuchiya K, Tattersall IW, Fotakis P, Bochem AE, Molusky MM, Ntonga V, Abramowicz S, Parks JS, Welch CL, et al. Deficiency of ATP-binding cassette transporters A1 and G1 in endothelial cells accelerates atherosclerosis in mice. *Arterioscler Thromb Vasc Biol*. 2016;36:1328–1337. doi: 10.1161/ATVBAHA.115.306670
8. Yvan-Charvet L, Pagler T, Gautier EL, Avagyan S, Siry RL, Han S, Welch CL, Wang N, Randolph GJ, Snoeck HW, et al. ATP-binding cassette transporters and HDL suppress hematopoietic stem cell proliferation. *Science*. 2010;328:1689–1693. doi: 10.1126/science.1189731
9. Yvan-Charvet L, Ranalletta M, Wang N, Han S, Terasaka N, Li R, Welch C, Tall AR. Combined deficiency of ABCA1 and ABCG1 promotes foam cell accumulation and accelerates atherosclerosis in mice. *J Clin Invest*. 2007;117:3900–3908. doi: 10.1172/JCI33372
10. Khera AV, Cuchel M, de la Llera-Moya M, Rodrigues A, Burke MF, Jafri K, French BC, Phillips JA, Mucksavage ML, Wilensky RL, et al. Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. *N Engl J Med*. 2011;364:127–135. doi: 10.1056/NEJMoa1001689
11. Rohatgi A, Khera A, Berry JD, Givens EG, Ayers CR, Wedin KE, Neeland IJ, Yuhanna IS, Rader DR, de Lemos JA, et al. HDL cholesterol efflux capacity and incident cardiovascular events. *N Engl J Med*. 2014;371:2383–2393. doi: 10.1056/NEJMoa1409065
12. Shea S, Stein JH, Jorgensen NW, McClelland RL, Tascau L, Shrager S, Heinecke JW, Yvan-Charvet L, Tall AR. Cholesterol mass efflux capacity, incident cardiovascular disease, and progression of carotid plaque. *Arterioscler Thromb Vasc Biol*. 2019;39:89–96. doi: 10.1161/ATVBAHA.118.311366
13. Costet P, Luo Y, Wang N, Tall AR. Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. *J Biol Chem*. 2000;275:28240–28245. doi: 10.1074/jbc.M003337200
14. Joseph SB, McKilligin E, Pei L, Watson MA, Collins AR, Laffitte BA, Chen M, Noh G, Goodman J, Hagger GN, et al. Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proc Natl Acad Sci USA*. 2002;99:7604–7609. doi: 10.1073/pnas.112059299
15. Kirchgessner TG, Sleph P, Ostrowski J, Lupisella J, Ryan CS, Liu X, Fernando G, Grimm D, Shipkova P, Zhang R, et al. Beneficial and adverse effects of an LXR agonist on human lipid and lipoprotein metabolism and circulating neutrophils. *Cell Metab*. 2016;24:223–233. doi: 10.1016/j.cmet.2016.07.016
16. Grefhorst A, Elzinga BM, Voshol PJ, Plösch T, Kok T, Bloks VW, van der Sluijs FH, Havekes LM, Romijn JA, Verkade HJ, et al. Stimulation of lipogenesis by pharmacological activation of the liver X receptor leads to production of large, triglyceride-rich very low density lipoprotein particles. *J Biol Chem*. 2002;277:34182–34190. doi: 10.1074/jbc.M204887200
17. Hong C, Marshall SM, McDaniel AL, Graham M, Layne JD, Cai L, Scotti E, Boyadjian R, Kim J, Chamberlain BT, et al. The LXR-Idol axis differentially regulates plasma LDL levels in primates and mice. *Cell Metab*. 2014;20:910–918. doi: 10.1016/j.cmet.2014.10.001
18. Xu Y, Liu C, Han X, Jia X, Li Y, Liu C, Li N, Liu L, Liu P, Jiang X, et al. E17241 as a novel ABCA1 (ATP-binding cassette transporter A1) upregulator ameliorates atherosclerosis in mice. *Arterioscler Thromb Vasc Biol*. 2021;41:e284–e298. doi: 10.1161/ATVBAHA.120.314156
19. Chen X, Zhao Y, Guo Z, Zhou L, Okoro EU, Yang H. Transcriptional regulation of ATP-binding cassette transporter A1 expression by a novel signaling pathway. *J Biol Chem*. 2011;286:8917–8923. doi: 10.1074/jbc.M110.214429
20. Thymiakou E, Zannis VI, Kardassis D. Physical and functional interactions between liver X receptor/retinoid X receptor and Sp1 modulate the transcriptional induction of the human ATP binding cassette transporter A1 gene by oxysterols and retinoids. *Biochemistry*. 2007;46:11473–11483. doi: 10.1021/bi700994m
21. de Haan W, Karasinska JM, Ruddle P, Hayden MR. Hepatic ABCA1 expression improves β-cell function and glucose tolerance. *Diabetes*. 2014;63:4076–4082. doi: 10.2337/db14-0548
22. Brunham LR, Kruit JK, Pape TD, Timmins JM, Reuwer AQ, Vasanji Z, Marsh BJ, Rodrigues B, Johnson JD, Parks JS, et al. Beta-cell ABCA1 influences insulin secretion, glucose homeostasis and response to thiazolidinedione treatment. *Nat Med*. 2007;13:340–347. doi: 10.1038/nm1546
23. Koseki M, Hirano K, Masuda D, Ikegami C, Tanaka M, Ota A, Sandoval JC, Nakagawa-Toyama Y, Sato SB, Kobayashi T, et al. Increased lipid rafts and accelerated lipopolysaccharide-induced tumor necrosis factor-alpha secretion in Abca1-deficient macrophages. *J Lipid Res*. 2007;48:299–306. doi: 10.1194/jlr.M600428-JLR200
24. Zhu X, Lee JY, Timmins JM, Brown JM, Boudyguina E, Mulya A, Gebre AK, Willingham MC, Hiltbold EM, Mishra N, et al. Increased cellular free cholesterol in macrophage-specific Abca1 knock-out mice enhances pro-inflammatory response of macrophages. *J Biol Chem*. 2008;283:22930–22941. doi: 10.1074/jbc.M801408200
25. Yvan-Charvet L, Welch C, Pagler TA, Ranalletta M, Lamkanfi M, Han S, Ishibashi M, Li R, Wang N, Tall AR. Increased inflammatory gene expression in ABC transporter-deficient macrophages: free cholesterol accumulation, increased signaling via toll-like receptors, and neutrophil infiltration of atherosclerotic lesions. *Circulation*. 2008;118:1837–1847. doi: 10.1161/CIRCULATIONAHA.108.793869
26. Westerterp M, Fotakis P, Ouimet M, Bochem AE, Zhang H, Molusky MM, Wang W, Abramowicz S, la Bastide-van Gemert S, Wang N, et al. Cholesterol efflux pathways suppress inflammasome activation, NETosis, and atherogenesis. *Circulation*. 2018;138:898–912. doi: 10.1161/CIRCULATIONAHA.117.032636
27. Bochem AE, van der Valk FM, Tolani S, Stroes ES, Westerterp M, Tall AR. Increased systemic and plaque inflammation in ABCA1 mutation carriers with attenuation by statins. *Arterioscler Thromb Vasc Biol*. 2015;35:1663–1669. doi: 10.1161/ATVBAHA.114.304959
28. Yu M, Amengual J, Menon A, Kamaly N, Zhou F, Xu X, Saw PE, Lee SJ, Si K, Ortega CA, et al. Targeted nanotherapeutics encapsulating liver X receptor agonist GW3965 enhance antiatherogenic effects without adverse effects on hepatic lipid metabolism in Ldlr(-/-) mice. *Adv Healthc Mater*. 2017;6:1700313.
29. Rayner KJ, Sheedy FJ, Esau CC, Hussain FN, Temel RE, Parathath S, van Gils JM, Rayner AJ, Chang AN, Suarez Y, et al. Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. *J Clin Invest*. 2011;121:2921–2931. doi: 10.1172/JCI57275
30. Rotllan N, Ramírez CM, Aryal B, Esau CC, Fernández-Hernando C. Therapeutic silencing of microRNA-33 inhibits the progression of atherosclerosis in Ldlr-/- mice–brief report. *Arterioscler Thromb Vasc Biol*. 2013;33:1973–1977. doi: 10.1161/ATVBAHA.113.301732
31. Rayner KJ, Esau CC, Hussain FN, McDaniel AL, Marshall SM, van Gils JM, Ray TD, Sheedy FJ, Goedeke L, Liu X, et al. Inhibition of miR-33a/b in non-human primates raises plasma HDL and lowers VLDL triglycerides. *Nature*. 2011;478:404–407. doi: 10.1038/nature10486
32. Price NL, Rotllan N, Zhang X, Canfrán-Duque A, Nottoli T, Suarez Y, Fernández-Hernando C. Specific disruption of Abca1 targeting largely mimics the effects of miR-33 knockout on macrophage cholesterol efflux and atherosclerotic plaque development. *Circ Res*. 2019;124:874–880. doi: 10.1161/CIRCRESAHA.118.314415
33. Price NL, Singh AK, Rotllan N, Goedeke L, Wing A, Canfrán-Duque A, Diaz-Ruiz A, Araldi E, Baldán Á, Camporez JP, et al. Genetic ablation of miR-33 increases food intake, enhances adipose tissue epansion, and promotes obesity and insulin resistance. *Cell Rep*. 2018;22:2133–2145. doi: 10.1016/j.celrep.2018.01.074