



ANGPTL3, PCSK9, and statin therapy drive remarkable reductions in hyperlipidemia and atherosclerosis in a mouse model¹

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The current focus of therapeutic intervention to reduce atherosclerotic cardiovascular disease (ACVD) risk is lowering plasma LDL-C to below 70 mg/dl using primarily statins. However, many patients on statins remain at high ASCVD risk (1). Monoclonal antibodies against PCSK9, in addition to statins, can further reduce LDL-C. Also, Mendelian randomization reports show that moderately elevated triglyceride-rich lipoproteins and remnant cholesterol increase ASCVD risk independently of LDL-C levels (2). This supports the use of drug combinations targeting complementary pathways explored in the study by Pouwer and collaborators (3) reported in this issue of the *Journal of Lipid Research*. This also suggests that simultaneous therapeutic reduction in plasma triglycerides associated with apoB-containing lipoproteins could have a greater atheroprotective effect (4). The consensus documents from the European Society of Atherosclerosis (EAS) and the International Society of Atherosclerosis (ISA) support the central role of apoB lipoproteins as a cause of ASCVD (1). The study by Pouwer et al. (3) in a preclinical mouse model and the human Mendelian randomization findings indicate that the contribution of LDL-C to the development of ASCVD is determined not only by the absolute LDL-C level but also by the cumulative time exposure of the arterial wall to apoB lipoproteins (1).

Pouwer et al. (3) report results from a study comparing double and triple combination treatments with the hypolipidemic drugs alirocumab and evinacumab, a human PCSK9 and an ANGPTL3 monoclonal antibody, respectively, combined with atorvastatin. In this interesting study, the effects on plasma lipoprotein levels and their impact on atherosclerosis progression and regression were evaluated in apoE*3Leiden.CETP mice, a well-established humanized model characterized by elevated triglycerides and VLDL-C remnant lipoproteins. This is the first mouse study using a combination of drugs currently in humans and in clinical

development for treatment of dyslipidemias and reduction of atherosclerotic cardiovascular risk.

Alirocumab is a monoclonal antibody to PCSK9 that reduces LDL-C plasma levels by $\geq 51\%$ and the risk of recurrent ischemic cardiovascular events in patients with acute coronary syndrome when administered with statins (5). Evinacumab (REGN1500), under clinical development, is a monoclonal antibody against ANGPTL3, a circulating protein secreted by the liver that regulates the hydrolysis of VLDL triglycerides (TGs) and HDL phospholipids by lipoprotein lipase and endothelial lipase, respectively (6).

In mice, inactivation of PCSK9 increases hepatic LDL receptors (LDL-Rs), consequently reducing circulating LDL levels (7). On the other hand, ANGPTL3 inactivation increases TG-rich VLDL hydrolysis, thus generating remnants apoB lipoproteins and their conversion into LDL particles rapidly cleared from circulation with or without direct involvement of hepatic LDL-R (8). The mice in this study received a Western-type diet containing 0.30% cholesterol and 15% saturated fat for 13 weeks that enhanced hyperlipidemia and accelerated atherosclerosis. The animals received the diet with different drug combinations for 25 weeks in the following groups: 1) control without any hypolipidemic drug, 2) atorvastatin as monotherapy, 3) atorvastatin with alirocumab, 4) atorvastatin with evinacumab, or 5) triple treatment with atorvastatin, alirocumab, and evinacumab. All drugs reduced total cholesterol (TC), nonHDL-C, and TGs with the triple treatment achieving the highest reduction at the end of the study. No changes in HDL-C were observed in any treatment.

This study confirmed that alirocumab and evinacumab, targeting different biochemical pathways together with a statin, caused an additive reduction of apoB-containing lipoproteins. Atorvastatin alone induced a 27% reduction, while the combination of atorvastatin with alirocumab or

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with evinacumab resulted in 53% and 47% reduction, respectively, and the triple treatment resulted in an additive 73% reduction in LDL-C. Remarkably, in VLDL-C, the most abundant plasma lipoprotein component in this model, the dual treatments showed a similar decrease in VLDL-cholesterol/remnant lipoprotein levels of 83% with alirocumab and 87% with evinacumab, compared with 45% with only atorvastatin, while the triple treatment increased the reduction to 93%. Surprisingly, PCSK9 inhibition with alirocumab decreased VLDL-C/remnant lipoprotein to a similar extent as evinacumab, the ANGPTL3 inhibitor. These results are consistent with previous human pharmacological studies showing that increases in hepatic receptors also could clear circulating remnant lipoproteins (9).

Pouwer et al. (3) show, for the first time, that combined alirocumab or evinacumab on top of atorvastatin completely blocks progression of preexistent atherosclerosis and that triple treatment regresses atherosclerosis. The impact of the different treatments on atherosclerosis progression and regression was quantified using the “time cumulative exposure” of plasma lipids of each mouse. The effects on lesion area were linearly predicted by the TC exposure reduction ($R = 0.85$; $P \leq 0.001$) and that of TG (0.64 ; $P \leq 0.001$). In addition, the atherosclerotic lesion regression showed that the cumulative exposure difference in plasma TC versus baseline contributed significantly to the model ($P < 0.001$), whereas the cumulative exposure difference in plasma TG versus baseline did not ($P = 0.193$). This clearly reinforced the key role of total apoB-lipoprotein cholesterol lowering (VLDL and LDL) in lesion regression ($R^2 = 0.72$) independently of TG.

Atherosclerotic plaque inflammatory markers and plaque composition are important features associated with clinical symptomatic lesions (10). PCSK9 deficiency in dyslipidemic mice decreases expression of endothelial chemotactic factors that promote monocyte adhesion and infiltration into the vessel (7). On the other hand, suppression of ANGPTL3 may induce local pro-inflammatory effects in the vascular wall by increasing endothelial lipase and lipoprotein lipase local activity (11, 12). In the present study, double and triple administration of the drugs decreased endothelial expression of ICAM-1, thus reducing monocyte adhesion to the vascular endothelium and appearing to improve markers of plaque stability to a similar extent. However, only the triple treatment reduced macrophage plaque content accompanied by a decrease in the number of Ki67-positive macrophages, a marker of proliferating macrophages. Membrane cholesterol cell accumulation and cholesterol crystals can activate macrophages (13). In this mouse model, the triple drug treatment showed the highest effect in plasma TC due to atherogenic apoB-lipoprotein reduction together with positive modulation of the inflammation-related cellular response. Two effects appear responsible for the athero-protective results observed. Thus, the valuable study by Pouwer et al. in the mouse model used suggests that rigorous apoB-lipoprotein-cholesterol reduction with drugs with complementary action mechanisms

may be an effective approach to significantly decrease atherosclerosis progress and induce regression in the presence of a Western diet.

The mechanism behind the observations in this study deserves further elucidation. First, evaluation of lipid and lipoprotein clearance could explain the marked reduction in TC. An enhanced VLDL and LDL uptake by the liver can cause hepatic intracellular cholesterol accumulation that, combined with statin treatment, may downregulate gene expression of proteins involved in normal cholesterol and fatty acid metabolism. Therefore, it is important to evaluate relevant gene expression analysis in the model and to measure liver and fecal sterol and bile acid content. Another aspect that merits further investigation is possible biochemical changes induced in lipoprotein structure by the combined treatments. Evaluation of particle size, surface charge, and apoprotein and lipid composition are parameters that could be behind the athero-protective effect observed. Their evaluation could be used as clinical biomarkers of the anti-atherosclerotic actions of the combined treatment.

The valuable study by Pouwer and collaborators, using the APOE*3-Leiden.CETP mouse, a well-established model for human combined dyslipidemia, supports the rationale for combined therapy for apoB lipoprotein reduction and atherosclerosis regression. Obviously, the eventual translation of these results into treatment of humans with these drug combinations will depend on clinical documentation of long-term safety and efficacy in ACVD risk reduction. 

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