Triglycerides and cardiovascular disease

Despite remarkable progress in the prevention of atherothrombosis through lowering of LDL cholesterol (LDL-C), considerable cardiovascular risk remains. Triglycerides have gained renewed recognition as a marker of residual risk in patients with atherosclerotic cardiovascular disease (ASCVD) and in those with cardiometabolic risk factors. Several studies have shown an independent association of elevated triglycerides with an increased risk of cardiovascular events, even among patients with low LDL-C (<1.8 mmol/L or <70 mg/dL) treated with high-intensity statin therapy. Furthermore, recent genome-wide association studies and Mendelian randomization analyses support a causal role for triglyceride-rich lipoproteins (TRLs) in events due to coronary artery disease (CAD). For example, carriers of mutations that result in lifetime low levels of triglycerides have reduced risk of CAD. These observations support targeting triglycerides for reduction of cardiovascular risk.

The recent Reduction of Cardiovascular Events with Icosapent Ethyl-Intervention Trial (REDUCE-IT) furnished further support for the triglyceride-lowering hypothesis. In REDUCE-IT, 8179 patients with established cardiovascular disease, or with risk factors, elevated triglycerides (>1.5 mmol/L or >135 mg/dL), and optimal LDL-C (1.0–2.6 mmol/L or 40–100 mg/dL) on background statin therapy randomly received 2 g twice daily of icosapent ethyl, a highly purified ethyl ester of eicosapentaenoic acid that lowers triglycerides, or placebo. Relative to placebo, icosapent ethyl decreased triglycerides by ~20%, the risk of ischaemic events including cardiovascular death by 25%, and total ischaemic events by 30% during the median follow-up of 4.9 years. Lowering of triglycerides probably contributed to the substantial benefit of icosapent ethyl in reducing ischaemic events in REDUCE-IT, in addition to other effects on prevention of atherothrombosis. Notably, most randomized clinical trials of drugs that lower triglycerides (i.e. fibrates, niacin, and omega-3 fatty acids except icosapent ethyl 4 g/day) have not shown reduction in cardiovascular events in statin-treated patients. Thus, despite the increased cardiovascular risk associated with triglycerides >1.1 mmol/L (>100 mg/dL), current guidelines only recommend pharmacological treatment for elevated triglycerides for severe hypertriglyceridaemia to prevent pancreatitis. Advancements in human genetics over the last decade have broadened our understanding of mechanisms of association of triglycerides with CAD. These developments have resulted in the discovery of novel pathways that regulate triglyceride levels, including apolipoprotein C3 (APOC3), angiopoietin-like 3, and angiopoietin-like 4, proteins currently under evaluation to lower triglycerides and reduce cardiovascular risk.

APOC3, triglycerides, and cardiovascular disease

APOC3 is a small apolipoprotein (79 amino acids) encoded by the APOC3 gene. Produced predominantly in the liver, APOC3 circulates attached to atherogenic TRLs such as very-low-density lipoprotein (VLDL), chylomicrons, and remnant cholesterol (Figure 1). Several studies have identified APOC3 as a key regulator of TRL metabolism. APOC3 increases levels of triglycerides by inhibiting the catabolism of TRLs by lipoprotein lipase and hepatic lipase, and by delaying remnant TRL clearance. Recent studies have associated loss-of-function variants in the APOC3 gene with decreased levels of APOC3, which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com
low triglycerides, and reduced risk of CAD.\textsuperscript{3,4} Elevated APOC3 levels are associated with an increased level of triglycerides, VLDLs, chylomicrons, remnant cholesterol, inflammation, and higher cardiovascular risk.\textsuperscript{13} Therefore, evidence thus far points to reduction of APOC3 as a promising therapeutic approach to lower triglycerides and reduce cardiovascular risk. To this end, an antisense oligonucleotide developed to target APOC3 mRNA lowers triglycerides by reducing APOC3 protein synthesis in the liver. As explained below, the advent of a selective strategy for targeting the liver in late-generation antisense oligonucleotides has markedly lowered the doses required to block the synthesis of proteins produced in hepatocytes and thus offers considerably improved tolerability of these agents.

**Antisense inhibition of APOC3**

Advances in genetic technology have enabled targeting of disease-associated mRNAs.\textsuperscript{14} Antisense oligonucleotides consist of nucleotides complementary to the mRNA encoding a protein of interest. If they can gain access to relevant cells, antisense oligonucleotides can seek their complement and bind to the target mRNA. Specialized RNases degrade such RNA:RNA duplexes, and prevent the production of the protein encoded by the mRNA. Accordingly, an APOC3 antisense oligonucleotide blocks the synthesis of APOC3 (Figure 1).

In this issue of the *European Heart Journal*, Alexander et al. report the results of safety and efficacy of hepatocyte-targeted antisense oligonucleotide-mediated inhibition of APOC3 mRNA in 67 healthy individuals with triglyceride levels of either $>1.0\text{ mmol/L (}>90\text{ mg/dL)}$ or $>2.3\text{ mmol/L (}>200\text{ mg/dL)}$.\textsuperscript{15} In this phase I study, subcutaneous administration of APOC3 antisense oligonucleotides in a single or multiple ascending dose approach resulted in a dose-dependent significant reduction in plasma APOC3 and triglyceride concentrations. Treatment with APOC3 antisense oligonucleotides decreased triglycerides by up to 70% and also reduced VLDL and apolipoprotein B (apoB), all of which have been implicated in atherosclerosis. The triglyceride-lowering effect of APOC3 inhibition persisted several weeks after the last dose (up to 4 months with dosing every 4 weeks). The APOC3 antisense oligonucleotide used in this study was well tolerated; the investigators noted one injection site reaction that did not result in drug discontinuation, and there were no deaths or other serious adverse effects.

The reduction in triglycerides with APOC3 inhibition in this study agrees with previous reports of antisense oligonucleotide targeting of APOC3 mRNA in patients with hypertriglyceridaemia including those with extremely high triglycerides.\textsuperscript{16,17} Prior APOC3 antisense oligonucleotide therapy did not lower apoB as seen in the present study. Volanesorsen, an earlier generation antisense oligonucleotide designed to inhibit APOC3 mRNA, reduced triglyceride levels by 60–70%. While a few patients receiving volanesorsen had declines in platelet count.\textsuperscript{18} no such concerns emerged with the APOC3 antisense oligonucleotide studied by Alexander and colleagues.

Recently, the development of antisense oligonucleotide technology for targeting disease-related RNAs has made substantial progress. To date, many antisense oligonucleotide-based therapies have received regulatory approval for treatment of diverse diseases including muscular dystrophy (e.g. eteplirsen), amyloidosis (e.g. inotersen), and hypercholesterolaemia (e.g. mipomersen), and many are being investigated in clinical trials.\textsuperscript{14} Because our clinical experience with RNA-based therapeutics is still in its infancy, they require particularly rigorous evaluation of safety. Several safety concerns regarding antisense oligonucleotides warrant meticulous monitoring in all future studies of APOC3 antisense oligonucleotides and other drugs that apply this technology.\textsuperscript{19} First, administration of antisense oligonucleotide may activate the immune system, leading to flu-like reactions, serum sickness, or even anaphylaxis. These immunological reactions may also generate antidrug antibodies and increase C-reactive protein levels. While a few patients receiving volanesorsen had immune reactions, there were none with the APOC3 antisense oligonucleotide in the present study. Secondly, many antisense oligonucleotides including volanesorsen have caused significant declines in platelet count. Thirdly, renal adverse events have been reported with a few antisense oligonucleotides. Finally, injection site reactions, the most frequent complication of oligonucleotide therapy, were encountered commonly with volanesorsen. Overall, the safety profile of the APOC3 antisense oligonucleotide reported here appears to be more promising than those of prior reports of volanesorsen. This progress could be due to conjugation of antisense oligonucleotide with a sugar moiety, N-acetyl galactosamine (GalNAc), which binds to a hepatocyte surface receptor for asialoglycoproteins. This novel approach offers efficient delivery to the liver, decreases extra-hepatic exposure, and requires a lower dose for the same therapeutic effect. Safety monitoring in a large number of patients and over a longer duration of time than examined in the present study is needed to draw any definitive conclusions about the safety profile of APOC3 antisense oligonucleotide therapy.

**Future directions**

We have entered an era of feasibility of targeting of APOC3 RNA to lower triglycerides using antisense oligonucleotides. This strategy might reduce the residual burden of cardiovascular events attributed to elevated triglycerides in patients with established cardiovascular disease and in those with risk factors (e.g. diabetes mellitus, insulin resistance, and obesity). Yet, whether lowering of triglycerides with APOC3 antisense oligonucleotides reduces cardiovascular events remains unknown. This approach is similar to those previously considered for the development of monoclonal antibodies against proprotease convertase subtilisin/kexin type 9 (PCSK9) for lowering LDL-C, a concept which emerged from genetic investigations as did recent studies for APOC3. Correspondingly, antibodies targeting APOC3 are also under development. Extrapolating from genetic studies, where an ~40% lower triglyceride level in carriers of loss-of-function variants in APOC3 translated to 40% reduced risk of coronary artery disease,\textsuperscript{20} a >50% reduction in triglycerides with APOC3 RNA inhibition appears promising for reduction of cardiovascular risk. Robust lowering of triglycerides with APOC3 antisense oligonucleotide may also prove beneficial in patients with very high triglyceride levels who have an increased risk of pancreatitis and who do not achieve optimal triglyceride levels with existing therapies.

Overall, advances in human genetics and biotechnology have spurred the identification of new targets and enabled the swift development of new therapeutics to address them. This union of basic science, applied technology, and clinical research has enabled
remarkable and rapid progress in combating cardiovascular risk. The major advance of the development of APOC3 antisense oligonucleotides illustrates the power of this multidisciplinary approach in addressing the ‘unmet need’ for therapies to lower the residual cardiovascular risk driven by elevated triglycerides. Physicians and patients alike will eagerly follow the journey of this therapy and allied novel therapies in future studies, ultimately leading to cardiovascular outcomes trials that will offer new inroads into fighting cardiovascular diseases.

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