Apolipoprotein C-III
Going Back to the Future for a Lipid Drug Target

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Interest in the relationship between plasma triglyceride (TG) concentration and cardiovascular disease (CVD) risk has recently resurged. Plasma TG concentrations in both the postprandial and fasting states are independent CVD risk factors and are associated with increased incidence of myocardial infarction and ischemic stroke. Furthermore, the dyslipidemias associated with insulin resistance is commonly characterized by increased plasma concentrations of TG-rich very-low-density lipoprotein (VLDL), which is considered a primary causal factor for the increased CVD risk in patients with the metabolic syndrome or type 2 diabetes mellitus. In addition, severely elevated TG concentrations caused by fasting chylomicronemia markedly raise the risk of acute pancreatitis. Effective treatments for elevated TG are sparse; their clinical efficacy varies, and evidence linking their use to protection from CVD has been difficult to obtain. A new therapeutic direction involves the regulation of gene expression. Thus, the development of antisense oligonucleotides (ASOs) that target apolipoprotein (apo) C-III, which is intimately involved in TG metabolism, represents a novel therapeutic approach. Although apo C-III has been known for >4 decades to be an important modulator of TG metabolism, a recent flurry of experimental data from animal models and human genetics has thrust it into the limelight as a promising target for the treatment of dyslipidemia and the prevention of atherosclerosis (Figure).

Apo C-III, Biochemistry, Lipoprotein Transport, and Intracellular Hepatic Function
Apo C-III is a small (79 amino acids) O-linked glycoprotein composed of 6 amphipathic α-helices that is expressed mainly in the liver and intestine. Plasma apo C-III concentration ranges from ~10 mg/dL in normal humans to >30 mg/dL in hypertriglyceridemic subjects. Its high affinity for lipoprotein surfaces positions apo C-III as a major protein component of chylomicrons (postprandially), as well as VLDL and high-density lipoprotein. On entry into plasma, apo C-III is rapidly exchanged among TG-rich lipoproteins and high-density lipoprotein. In plasma, apo C-III attenuates the activity of lipoprotein lipase and interferes with receptor-mediated and receptor-independent clearance of TG-rich lipoproteins. After lipolysis of core TG, apo C-III is transferred to high-density lipoprotein, which serves as a plasma reservoir for this apolipoprotein. From there, apo C-III is poised to rapidly transfer onto newly secreted TG-rich lipoproteins. Apo C-III is thought to delay premature receptor-mediated clearance of TG-rich lipoproteins until a sufficient quantity of TG-derived fatty acid is taken up by peripheral tissues.

An intrahepatic role for human apo C-III, namely the promotion of VLDL assembly and secretion, was recently discovered. Overexpression of human apo C-III, either in cultured hepatocytes or in apo C-III transgenic mice, stimulated the assembly and secretion of TG-rich VLDL under lipid-rich conditions. These studies showed that apo C-III recruits TG during the late stages of VLDL assembly, thus relieving the hepatocyte of excess TG. Apo C-III harboring either of 2 known loss-of-function mutations was incapable of performing this function. An interesting interpretation of this totality of evidence is that increased hepatic apo C-III production stimulates VLDL secretion as a hepatic protective mechanism, preventing steatosis in the context of increased hepatic fatty acid flux.

Elevated plasma apo C-III concentrations also are closely correlated with hypertriglyceridemia in mice and humans. Increased apo C-III levels in humans with hypertriglyceridemia are tied to both increased production and decreased catabolism of apo C-III. Elevated VLDL apo C-III concentration is strongly and independently predictive of coronary heart disease, even more than TG alone. Also, LDL that contains apo C-III, produced by partial lipolysis in plasma of apo C-III-containing VLDL, is highly predictive of CVD in type 2 diabetes mellitus.

Importance of Apo C-III From Genetics
Genetic studies in humans and in animal model systems confirm the central role of apo C-III in lipoprotein metabolism and vascular biology. The APOC3 gene resides within the important APOA5-APOA4-APOC3-APOA1 multigene cluster on human chromosome 11q23. This locus produces the strongest association signal in genome-wide association studies of plasma TG concentration, although there is some ambiguity about the particular genes and variants that underlie this signal. Although APOC3 was historically considered the strongest candidate gene locus associated with plasma TG concentration, the Global Lipids Genetics Consortium investigators and others working with genome-wide association studies have more recently designated...
APOA5 as the source of the association signal. However, there are complex patterns of linkage disequilibrium between common polymorphisms of APOA5 and APOC3, and it remains possible that either gene, both in concert, or perhaps an entirely different gene at the locus is the mechanistic basis for the association signal with plasma TG levels. Importantly, polymorphisms at this locus also have been associated with atherosclerosis end points in genome-wide association studies.

Two common functional variants in the APOC3 promoter region, namely 482C>T and 455T>C, attenuate the normal reduction of APOC3 expression in response to insulin, resulting in increased apo C-III expression in the context of hyperinsulinemia. In other words, these variants create a state of insulin resistance at the molecular level. These APOC3 variants were also associated with a 2-fold increase in fasting plasma TG concentration in Asian Indian men and with increased risk of metabolic syndrome in multiethnic samples.

Some of the most compelling evidence favoring apo C-III as a target for metabolism and atherosclerosis derives from studies of rare large-effect genetic variants in APOC3. For example, a heterozygous nonsense mutation in APOC3 at residue 19 (R19X) was found in 2.8% of Amish subjects. In R19X carriers, plasma apo C-III concentrations were reduced by 50% compared with noncarriers. The R19X variant was further associated with decreased plasma TG and LDL cholesterol and with increased plasma high-density lipoprotein cholesterol. Subclinical atherosclerosis, as measured by electron beam computed tomography, also was significantly reduced in carriers. Another rare APOC3 variant, the alanine-to-threonine missense mutation at residue 23 (A23T), was identified in 3 Yucatan Indian subjects with apo C-III deficiency. Functional characterization of A23T in vitro demonstrated attenuated VLDL assembly and secretion from hepatocytes, with TG accumulation within the microsomal lumen that was independent of microsomal TG transfer protein activity.

The above studies suggested that pharmacological inhibition of APOC3 could improve elevated plasma TG phenotypes. The putative biochemical functions of apo C-III are further consistent with in vivo model system studies showing hypertriglyceridemia in mice overexpressing human APOC3 and hypotriglyceridemia and enhanced postprandial TG clearance in Apoc3-deficient mice. All in all, the human and animal genetic data strongly suggest that reduction of apo C-III would be associated with an improved lipoprotein profile and likely reduced atherosclerosis, thus nominating apo C-III as a desirable therapeutic target.

Hitting the Target With ASOs
In the current issue of Circulation Research, Graham et al describe the development and efficacy of rodent- and human-specific second-generation ASOs that target apo C-III expression in mice, rats, and monkeys, as well as normal human volunteers (Figure). Administration of apo C-III ASOs in the animal models decreased hepatic apo C-III mRNA, plasma apo C-III, and plasma TG primarily in VLDL. Postprandial TG concentrations were also attenuated in treated mice. Apo C-III ASOs did not affect rates of TG secretion into plasma, nor did they increase liver TG in the various mouse models tested. Specificity for apo C-III was established in experiments showing no effect on TG levels in apo C-III ASO-treated Apoc3-deficient mice. The authors inferred that treatment induced increased clearance of TG-rich lipoproteins.

After receipt of multiple doses of apo C-III ASOs for >22 days, plasma apo C-III and TG concentrations trended lower in a relatively small number of normal humans, an effect that lasted another 28 days. Apo C-III ASO treatment was well tolerated, with no evidence of hepatotoxicity or other safety signals. This study represents an important advance in the search for therapeutics for hypertriglyceridemia. It brings to light the substantial and successful preclinical development of a molecular approach to lower apo C-III and presents the first phase I trial of this target in healthy human subjects. Finally, the data clearly support the authors’ conclusions that the ASO reduces plasma apo C-III and TG, both of which are CVD risk factors.

Some Questions for the Present
Despite the expected and internally consistent effects, the authors were unable to fully define the mechanism(s) linking apo C-III ASO treatment to the reduction in plasma TG. Specifically, rates of lipolysis and particle clearance were not assessed. These parameters are of central importance because hepatic apo C-III potentially promotes the clearance of excess hepatic TG. Therefore, ASO inhibition of apo C-III could theoretically exacerbate hepatic lipid accumulation. The authors did show that liver TG is significantly increased in Apoc3-deficient mice and trended to higher levels in apo C-III ASO-treated mice. Hepatic steatosis is seen in many patients with hypertriglyceridemia who might be eligible for apo C-III ASO treatment. Further development of this agent should include close attention to hepatic steatosis.

In wild-type mice, the impact of apo C-III ASOs on postprandial TG concentrations was much lower than the effect in apo C-III null mice, in which postprandial lipemia was very low. The authors suggested that because apo C-III ASOs primarily target the liver, suppression of intestinal apo C-III expression is relatively modest in treated wild-type mice, whereas intestinal apo C-III is completely absent in knockout mice. This would imply that apo C-III treatment may be less effective in patients whose hypertriglyceridemia was composed of elevated TG-rich lipoproteins of intestinal origin.

A primary therapeutic objective of apo C-III ASO treatment is to reduce CVD risk. Therefore, it is unfortunate that protection from atherosclerosis was not examined in the susceptible murine models under study. Although such studies are likely forthcoming, the regulatory approval and acceptance of this agent as a therapeutic modality may eventually depend on data implicating a cardioprotective role for apo C-III ASO treatment.

Longer-term studies are ultimately required to determine the efficacy and potential adverse effects of apo C-III ASO therapy. However, experience with the apo B ASO treatment mipomersen can provide clues to some issues that might arise. Like the apo C-III ASO, mipomersen has clear biochemical efficacy: it lowers LDL cholesterol by ≥50%. Because mipomersen inhibits hepatic apo B–containing lipoprotein secretion, this treatment in hypercholesterolemic subjects...
also decreased plasma apo C-III by ≈40%.33 Furthermore, mipomersen is effective in patients with homoyzglyous familial hypercholesterolemia, which is perhaps the most difficult to treat of all human monogenic dyslipidemias.32 In fact, its efficacy in LDL cholesterol reduction was central in convincing US Food and Drug Administration reviewers to recommend approval of mipomersen for the treatment of homoyzglyous familial hypercholesterolemia, despite the lack of CVD outcome data coupled with its frequent adverse effects, including annoying skin reactions at the site of injection, flu-like symptoms, and hepatosteatosis. The first 2 adverse effects seem to be related to the ASO platform in general, not specifically to the molecular target.34 Hepatosteatosis, on the other hand, is a predictable consequence of the targeting of apo B for suppression. Thus, by analogy with mipomersen, skin reactions and flu-like symptoms may be longer-term toxicities of apo C-III ASO therapy. Furthermore, hepatosteatosis might also prove to be a consequence of this treatment, given the experimental evidence presented above. However, as the approval of mipomersen demonstrated, if a new agent has substantial efficacy, this could offset concerns over the potential adverse effects, depending on the overall benefit-to-risk ratio. In the case of apo C-III ASO therapy, however, TG remains a less compelling target for CVD prevention than LDL cholesterol. It is thus unlikely that apo C-III ASO treatment would be approved on the basis of mere sustained biochemical benefit, without direct evidence for a vascular (or perhaps even pancreatitis-related) beneficial outcome with respect to morbidity and mortality.

**Back to the Future Again**

The report by Graham et al11 thus provides a first-in-human study demonstrating short-term efficacy and apparent safety of the apo C-III ASO approach. Definitive longer-term evidence of sustained and generalizable efficacy together with negligible adverse effects will be crucial to ensure that this treatment eventually sees the light of day. But although interesting and important metabolically, TG does not currently represent as persuasive a target as LDL cholesterol because of the longstanding controversy about the direct role of TG in CVD pathogenesis. Future genetic and epidemiological studies might clarify this risk and may well re-energize TG as a key CVD biomarker. Assuming that TG’s star continues to ascend, new therapies based on a wide swath of experimental data that justify targeting TG may represent smart biological antiatherosclerosis therapy of the future.

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None.

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