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EDITORIAL COMMENT

Lipoprotein(a)

An Enigmatic Sheep in the Lipoprotein Herd*

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Hypercholesterolemia is the principal risk factor that drives initiation and development of atherosclerotic cardiovascular disease (ASCVD), the leading cause of death and disability worldwide. Many individuals with hypercholesterolemia do not achieve adequate low-density lipoprotein-cholesterol (LDL-C) reduction with standard lipid-lowering therapies (e.g., statins) or are unable tolerate them. In 2003, the discovery of proprotein convertase subtilisin kexin type 9 (PCSK9), a low abundance plasma protein with a disproportionately large effect on cholesterol metabolism and plasma LDL-C concentration, ushered in a new era of physiological understanding and therapeutic potential. The development of therapeutic anti-PCSK9 monoclonal antibodies (e.g., PCSK9 inhibitors) transformed our ability to manage patients with ASCVD and familial hypercholesterolemia (FH).

The U.S. Food and Drug Administration initially approved the use of PCSK9 inhibitors based on their LDL-C lowering efficacy and safety while respective large cardiovascular outcomes trials were ongoing. Both therapeutic antibodies target the same region of PCSK9 and have similar LDL-C lowering efficacy (~60% reduction in LDL-C) at maximum doses. The results of cardiovascular outcome trials have been similarly impressive for both evolocumab and alirocumab; thus, the PCSK9 inhibitor class is now endorsed by many international guidelines for use in select patient populations (1).

One of the interesting and unanticipated facets of PCSK9 inhibition is its consistent association with the lowering of plasma lipoprotein (a) [Lp(a)] levels. Lp(a) is an enigmatic atherogenic lipoprotein that consists of an LDL-like particle with a protein constituent [apolipoprotein(a)] covalently bound to its apolipoprotein B moiety. A recent meta-analysis of 27 randomized controlled clinical trials that enrolled 11,864 subjects demonstrated significant and comparable reductions in Lp(a) with either PCSK9 inhibitor treatment (on average: −21.9%) (2). The mechanism(s) that underlie PCSK9 inhibitor associated reductions in plasma Lp(a) concentration remain unclear, although several hypotheses have been put forward, including: 1) enhanced Lp(a) clearance through the LDL receptor (LDLR) pathway; 2) enhanced Lp(a) clearance via other receptors (LDLR-related protein 1[LRP1], cluster of differentiation 36 receptor [CD36], toll-like receptor 2 [TLR2], scavenger receptor-B1 [SR-B1], and plasminogen receptors); and 3) reduction in apolipoprotein (a) production, secretion, and/or assembly to form Lp(a) particles.

Of the previously described, the most widely held view linking PCSK9 inhibition and Lp(a) reduction relates to enhanced LDLR-mediated clearance. However, the notion that Lp(a) clearance is mediated by LDLR poses several challenges: 1) Lp(a) has poor affinity for LDLR, far less than that of LDL (3); 2) the catabolic rate of Lp(a) is similar in subjects with FH and without FH; 3) Lp(a) levels are largely unaffected

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by other therapies that upregulate the LDLR (e.g., statins, ezetimibe) (4); 4) PCSK9 inhibition in patients with homozygous FH and null LDLR mutations lowers Lp(a) more than does LDL-C levels; 5) similar levels of Lp(a) were observed in carriers versus noncarriers of loss-of-function mutations in *PCSK9* (5,6); and 6) there is no consistent correlation between plasma PCSK9 and Lp(a) concentrations across epidemiological studies.

Regardless of mechanism, the epidemiological and genetic associations of Lp(a) with ASCVD and calcific aortic stenosis drive continued interest in understanding how PCSK9 inhibition may play a role in reducing the burden of Lp(a) associated disease. Moreover, recent focused subanalyses from the FOURIER (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk) and ODYSSEY OUTCOMES (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) trials lend credence to the notion that PCSK9 inhibitor–induced Lp(a) reduction may effectively reduce residual ASCVD risk (7,8). The findings from these subanalyses, with respect to PCSK9 inhibitor–associated Lp(a) lowering, are noteworthy and beg the question as to the potential future role of PCSK9 inhibition in thwarting residual cardiovascular risk in subjects with established ASCVD and elevated Lp(a), regardless of LDL-C.

With this as a backdrop, a timely mechanistic study by Chemello et al. (9) published in this issue of *JACC: Basic to Translational Science* gets to the heart of the question: does the LDLR contribute to Lp(a) clearance from plasma? The investigators conducted elegant experimental work in a murine model in which the host liver parenchyma was ablated and replaced with human hepatocytes under a near-normal architecture (mice with humanized liver). The mice were then treated with either alirocumab or placebo, and hepatic capture of fluorescent LDL and Lp(a) was assessed. The investigators found significant plasma LDL-C and Lp(a) lowering in the animals that received alirocumab compared with placebo. However, although alirocumab was associated with a significant increase in fluorescent LDL uptake by the human liver cells, there was no significant impact on fluorescent Lp(a) capture by these hepatocytes, thus suggesting a differential mechanism for the lowering of these 2 apolipoprotein B–containing particles from plasma. Similarly, the investigators performed parallel experiments evaluating cellular uptake of LDL and Lp(a) in primary lymphocytes isolated from normal subjects and from a patient with homozygous FH (absent LDLR function). The lymphocytes were incubated sequentially with or without mevastatin, recombinant PCSK9, or alirocumab. They found that fluorescent LDL cellular uptake followed the patterns of LDLR cell surface expression. In contrast, cellular uptake of fluorescent Lp(a) was similar in control and homozygous FH lymphocytes and was not affected by statin, PCSK9, or alirocumab treatments. In aggregate, these series of observations indicate that the LDLR does not play a major physiological role in clearance of Lp(a) because modulation of LDLR expression either genetically or pharmacologically failed to materially alter the cellular uptake of Lp(a) ex vivo or hepatic capture in vivo (4). In line with these experimental findings, the investigators' previous work suggested that PCSK9 influences apolipoprotein(a) synthesis and/or its assembly into Lp(a), mechanisms clearly independent of the LDLR pathway (10).

The basic science examined in this study provides mechanistic support for the empirical evidence we have had for years, namely, that statins lower plasma LDL-C by upregulating the expression of LDLR on hepatocytes without reduction in plasma Lp(a) concentration. Nevertheless, the consistent reductions in Lp(a) observed in all the PCSK9 inhibitor trials reinvigorated the debate regarding the relative role of LDLR in Lp(a) catabolism. However, a series of recent studies further corroborated the results of the study examined here. We previously hypothesized that if the LDLR was a major pathway for Lp(a) clearance, then inhibition of PCSK9 should produce proportionate reductions in LDL-C and Lp(a) in each subject, with an average approximating the 2:1 ratio (LDL-C ≈50% to 60%: Lp(a) ≈ 25% to 30%) seen in large randomized clinical trials. Results from our recent work highlighted that a significant proportion of patients actually demonstrate discordant responses in LDL-C and Lp(a) to PCSK9 inhibition, showing robust reductions in LDL-C but minimal or no reduction in Lp(a) (11,12). We performed an analysis of the PROFICIO (Program to Reduce LDL-C and Cardiovascular Outcomes Following Inhibition of PCSK9 in Different Populations) clinical trial program, evaluating 895 patients who received evolocumab. Baseline LDL-C and Lp(a) values were 133 and 46 mg/dl, respectively, with average reductions of 63.3% and 29.6% with evolocumab administration, which again confirmed the expected 2:1 ratio. The study demonstrated moderate correlation ($r = 0.37$; $p < 0.001$) between percent LDL-C and Lp(a) reduction. Discordance was progressively more prevalent among those with higher baseline Lp(a), >10 mg/dl (19.7%), >30 mg/dl (26.5%), and >50 mg/dl (28.6%). Recently, we performed a pooled analysis of 10 randomized

controlled trials from the ODYSSEY Phase III clinical trial program, which included patients at high cardiovascular risk and/or with FH. Once again, a high rate of discordance (22%) was observed between LDL-C and Lp(a) reduction with alirocumab and was independent of FH status (13). Importantly, both studies suggested there were other mechanisms and/or pathways beyond LDLR that accounted for reductions in Lp(a) levels induced by PCSK9 inhibitors.

Although there is no immediate clinical translation to these findings, they do provide the impetus to identify other potential mechanisms that govern the interaction(s) between PCSK9 and Lp(a), and the mysteries of Lp(a) assembly, secretion, processing, and clearance. Because PCSK9, and by extension, PCSK9 inhibitors, affect many receptors beyond the LDLR (e.g., APOER2, LRP1, VLDLR, CD36, TLR2, plasminogen receptors), it is conceivable that a PCSK9-controlled Lp(a) receptor may direct exit of Lp(a) from the plasma compartment. The fact that the Lp(a) lowering induced by PCSK9 inhibitors is related to baseline Lp(a) concentration suggests that Lp(a) clearance may be dependent on apolipoprotein (a) isoform size. The LDL-C and/or Lp(a) discordance observed in clinical studies may be due to clearance arbitrated by apolipoprotein (a) isoform size and not by the apolipoprotein B side of the lipoprotein. In this scenario, apolipoprotein (a) isoform size caused by genetic variation in the length of the kringle 4 type 2 chain may act as a major determinant of the ability of Lp(a) to clear the circulation via LDLR versus alternative receptors.

Large gaps remain in our understanding of PCSK9 physiology and function and in how antagonism of PCSK9 induces reduction of plasma Lp(a) levels. The mechanistic study by Chemello et al. (9) provides some clues to the biology of Lp(a) removal from circulation, an issue that has remained unresolved since the discovery of this unique lipoprotein in 1963. Based on this work and other corroborative evidence, we should move beyond the trending notion that Lp(a) is simply cleared by the LDLR pathway. However, even with this step forward, Lp(a) remains the enigmatic lipoprotein particle that the scientific community strives to figure out.

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