PCSK9 INHIBITION AND LP(A) REDUCTION: ANOTHER PIECE OF THE PUZZLE?

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Lipoprotein(a) [Lp(a)] is an LDL-like particle in which apolipoprotein(a) is linked to apoB via a disulfide bond; epidemiologic and genetic studies indicate that elevated plasma levels of Lp(a) are a cardiovascular risk factor independent of LDL[1]. Furthermore, a strong association between elevated Lp(a) levels and calcific aortic valve stenosis has been shown, and Mendelian randomization studies have confirmed that Lp(a) is causally involved[2]. Owing the sequence homology with plasminogen, high Lp(a) levels are also believed to promote an athero-thrombotic condition via several mechanisms, including the inhibition of the fibrinolytic system and enhancement of tissue factor-mediated pathway[3]. Finally, it is now clear that elevated Lp(a) levels remain a cardiovascular risk factor even in patients with controlled LDL-C levels[4], indicating that lowering Lp(a) levels should translate into a cardiovascular benefit.

Lp(a) levels are genetically determined, and primarily controlled by synthesis rather than catabolism[4], and the fact that statins, despite their efficacy in reducing LDL-C levels by increasing the hepatic LDLR expression, have no or little effect on Lp(a) levels,[5] and that lipid-lowering drugs which act by reducing apoB synthesis or LDL assembly (including mipomersen and lomitapide) reduce Lp(a) levels[6] was somehow in line with expectations. It has therefore become quite puzzling that PCSK9 inhibitors, which also act by increasing LDLR expression, reduce significantly Lp(a) levels up to 30%[7, 8].

Kinetic studies are believed to be the gold standard in understanding whether a pharmacological intervention modifies the rate of synthesis or catabolism of a given protein, and this approach has successfully elucidated *in vivo* the mechanisms by which statins and other drugs affecting plasma lipoproteins act[9, 10]. One of the complexities with lipoproteins is that the main protein of LDL, apoB, is mostly released in the circulation in VLDL and then eventually, by several remodelling passages, becomes a LDL; interpretation of the data via modelling of the kinetics is a must, with multiple compartments to fit the kinetic curves. A second level of complexity relates to Lp(a) owing the fact that the lipoprotein is assembled by associating to a LDL, making kinetic studies even more complex.

In the present issue, Watts and colleagues have investigated by kinetic studies the mechanisms by which evolocumab (a PCSK9 inhibitor) reduces Lp(a) levels[11]. They confirmed that the treatment with atorvastatin alone did not affect Lp(a) levels, with no changes in the fractional catabolic rate (FCR) or the production rate (PR) of Lp(a)-apo(a). Accordingly, the treatment with evolocumab or evolocumab+atorvastatin resulted in comparable reductions of Lp(a) levels (-33% and -38%, respectively)[11]. However, such reductions were achieved through two different mechanisms: when administered as monotherapy, evolocumab reduced the PR of Lp(a)-apo(a) without affecting its FCR[11]. In contrast, when given in combination with atorvastatin, the FCR of Lp(a)-apo(a) significantly increased, without alterations of its PR[11]. A previous study in which alirocumab was tested versus placebo reported different results: inhibition of PCSK9 reduced plasma Lp(a) levels by 18.7% ($p<0.01$), and this reduction was associated with a trend for an increase in the median FCR of Lp(a)-apo(a) (24.6%; $P=0.09$) and no changes in its PR[12]. The reason for this discrepancy is unclear; it should be noticed that the degree of Lp(a) reduction in this study was somewhat less[12]. Differences in the baseline

characteristics of subjects included in these two studies, including differences in Lp(a) baseline levels, age, BMI, and ethnicity[11, 12] may contribute in explaining these differences. Apo(a) isoform size may also play a role, as it may influence both production and catabolism of Lp(a) particles[13].

In vitro, both LDL and Lp(a) compete with LDL for the binding to LDLR, but higher concentrations of the latter are required, suggesting that the two lipoproteins have different affinities for LDLR, being that of LDL higher. Thus, it can be assumed that, when LDL-C levels are massively reduced, Lp(a) clearance can increase due to a higher availability of "free" LDLR. Statin-induced inhibition of intracellular cholesterol synthesis by inducing the activation of SREBP-2, upregulates the expression of both LDLR and PCSK9 and the expression of LDLR receptor might be not high enough to support direct Lp(a) removal. By analogy in the presence of PCSK9 inhibitors, circulating PCSK9 is reduced, leading to a higher availability of LDLR for LDL internalization, which again may not be enough to efficiently remove Lp(a) particles. However, under these circumstances, evolocumab reduced Lp(a) levels by reducing the production of Lp(a)[11]. This observation is supported by a previous study showing that PCSK9 enhanced the secretion of Lp(a) from cultured hepatocytes, an effect that was blunted by alirocumab, without any effect on Lp(a) uptake[14]. This study could not demonstrate an involvement of LDLR in the uptake of Lp(a)[14]. It is intriguing to suggest an intracellular role for PCSK9 in modulating Lp(a) plasma levels; the mechanism, however, has not been addressed so far. When given in combination with a statin, the concomitant increase of LDLR expression and reduction of circulating PCSK9 leads to a further increased availability of "free" LDLR and to a massive decrease of LDL-C; this leads to a profound reduction of the high affinity ligand of LDLR (i.e. LDL), thus allowing the binding of a lower-affinity ligand (i.e. Lp(a)). This finding is supported by the observation that the reduction in Lp(a) levels was significantly correlated with reduction in LDL-C levels: there was a greater Lp(a) percent reduction in patients who achieved LDL-C ≤40 mg/dL than in those who achieved LDL-C >70 mg/dL, supporting a relevant role of LDLR in the removal of Lp(a)[7]. One way to address this possibility would be to externally clamp LDL levels to higher values and then re-perform kinetics. A reduction of the FCR should be observed.

Alternative explanation may be based on the fact that experimental and clinical evidence suggests the involvement of additional receptors/pathways in the clearance of Lp(a)[15]. Evolocumab, although did reduce LDL-C levels only marginally in 2 HoFH patients carrying complete loss-of-function LDLR mutations, reduced significantly their Lp(a) levels despite the absence of LDLR[16], indicating the possible involvement of other pathways perhaps also intracellular. Some additional receptors have been suggested as likely involved in Lp(a) clearance; among them, the two members of the LDLR family VLDLR and megalin/LRP2[15]; plasminogen receptors, scavenger receptor type B class I (SR-BI) and sortilin have been proposed as well[15, 17]. The role of these receptors in the catabolism of Lp(a) is still largely unexplored, as it is the possible involvement of PCSK9 in regulating their expression. An alternative explanation that deserves consideration

is that PCSK9 also binds to Lp(a)[18], one could speculate that, in statin-treated patients, the increased amount of circulating PCSK9 leads to higher percent of Lp(a)–PCSK9 complexes, which in turn are recognized by the antibodies, promoting an alternative antibody driven removal pathway

Finally a word of caution on the methodology, although the careful nature of the work is appreciated, issues about the methodology and data analysis are still present. Isolating apo(a) and Lp(a) apo B is not an easy task and the studies of isotopic enrichment clearly depend on these processes; further the theoretical modeling with the different compartments also heavily depends on these data and small changes may preferentially favor a pathway *vs* another.

In summary to date, the mechanisms by which PCSK9 inhibition reduces Lp(a) levels are unclear, and the conflicting results reported in the studies of Watts[11] and Reyes-Soffer[12] confirm the complexity of Lp(a) metabolism and the fact that biology in not as simple as we tend to believe, several regulatory pathways can be in place and dissecting their relative role quite challenging. Clearly we need future studies addressing those aspects to understand, by the use of appropriate *in vitro* and *ex vivo* experiments, the complex picture that kinetic studies suggest to us.

Figure 1. Possible mechanisms of statins and mAbs to PCSK9 on Lp(a) metabolism. LDLR seems to be involved in Lp(a) catabolism, but other receptor may also play a role. (A) Under physiological conditions, LDLR surface expression is regulated by the content of intracellular cholesterol and the amount of extracellular PCSK9. (B) Statins reduce cholesterol biosynthesis, leading to the upregulation of both LDLR and PCSK9. LDL-C levels are decreased. Under this condition, LDLR is not available for a lower affinity binding with Lp(a) as it is mainly engaged in the removal of LDL. Other receptors possibly involved in Lp(a) uptake might be controlled by PCSK9. The increased levels of PCSK9 may lead to an increased formation of PCSK9-Lp(a) (and/or LDL) complexes. (C) In the presence of anti-PCSK9 mAb, extracellular PCSK9 is sequestered; thus, LDLR is recycled to the surface and available for new binding. The reduction of PCSK9 reduces the production of Lp(a) particles through several mechanisms. Lp(a)–PCSK9 complexes may be recognized by the anti-PCSK9 mAb, promoting an alternative removal pathway. (D) In the presence of statin and mAb to PCSK9, both LDLR expression and recycling are increased, leading to a massive reduction of LDL particles. This increases the number of free LDLR, which are thus available for the low affinity binding with Lp(a), leading to an increased Lp(a) uptake. Other receptors may contribute to the Lp(a) removal.

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