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Lipoprotein(a) and PCSK9 inhibition: clinical evidence

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Compelling evidence has emerged from epidemiological and Mendelian randomization analyses relative to the causality of lipoprotein(a) [Lp(a)] in atherosclerotic cardiovascular diseases (ASCVD), being elevated Lp(a) a strong risk factor regardless of the reduction of LDL-C achieved by statins. So far, no specific available agent can lower Lp(a) to the extent required to achieve a cardiovascular (CV) benefit, i.e. approximately 100 mg/dL. The most recent outcomes trial FOURIER with evolocumab showed that a 25 nmol/L (12 mg/dL) reduction in Lp(a) corresponded to a 15% decrement in the relative risk of cardiovascular disease. The ODYSSEY OUTCOMES trial with alirocumab has been the first demonstrating that a reduction in Lp(a) associates with less major adverse cardiovascular events (MACE), i.e. hazard ratio: 0.994 per 1 mg/dL decrement in Lp(a). The Lp(a) lowering effect driven by PCSK9 inhibition was confirmed in carriers of *PCSK9* loss-of-function mutations in which Lp(a) and oxPL-apoB levels were decreased compared to non-carriers as was for a slight larger number of apo(a) Kringle IV repeats. Although PCSK9 inhibitors are not able to decrease Lp(a) to the extent required to achieve a CV benefit, their use has led to a higher discontinuation rate in lipoprotein apheresis in patients with progressive ASCVD and high plasma Lp(a).

Introduction

Over the last decade, there has been an increasing interest and a rapidly evolving knowledge on lipoprotein(a) [Lp(a)], a revival essentially driven by genetic studies. Indeed, studies in animal models are scanty, since Lp(a) is only found in humans, some old-world monkeys, and European hedgehogs. Lp(a) consists of an apolipoprotein B-100 particle attached to apolipoprotein A by a disulphide bridge.[1] Apo(a) is a large protein encoded by *LPA* and it is composed of a signal peptide region, many repeating kringle domains, and a protease domain. In particular, Lp(a) contains multiple identical Kringle IV type 2 repeats (KIV2), being the number of apo(a) KIV2 repeats inversely proportional to plasma Lp(a) levels.[2] However, all studies that have used polymerase chain reaction (PCR) to quantitate the number of KIV2 repeats have limitations. The allele-specific numbers of KIV2 repeats cannot be determined by PCR, since data are reported as a summary of both alleles, e.g. total of 60 repeats could be due to a patient carrying 30 KIV2 repeats on each allele.[1] In this scenario, another important concern has been raised by some authors who compared in 144 serum samples six widely used commercially available assays for measuring Lp(a). This study shows absolute differences in Lp(a) measurements for single samples between two assays of up to almost 80 mg/dL. However, as a comparator the author did not use a gold standard assay which is able to measure Lp(a) in molar terms by using antibodies directed against a unique structure of apo(a) or by a mass-spectrometric approach.

In the context of cardiovascular (CV) risk, the relationship between Lp(a) and atherosclerosis has been unravelled by Mendelian Randomization and genome-wide association studies which showed that *LPA* genotype significantly

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associates with cardiovascular disease (CVD). High levels of Lp(a), corresponding to low *LPA* KIV2 number of repeats, have been associated with high risk of mortality.[1] This evidence has been corroborated in epidemiological studies suggesting that raised Lp(a) levels are a strong risk factor of atherosclerotic CVD (ASCVD) and by large clinical trials reporting that elevated Lp(a) remains a risk factor despite the reduction of LDL-C with statins. Conversely, a Mendelian randomization analysis concluded that a reduction in Lp(a) concentrations of approximately 100 mg/dL had an equivalent association with the coronary heart disease (CHD) risk reduction found for every 38.67 mg/dL difference in LDL-C (−22%). These results may explain why previous trials leading to Lp(a) reductions of only 20-30% failed to show an association between Lp(a) lowering and CV prevention.[3] Moreover, people with extremely high Lp(a) levels >180 mg/dL (>430 nmol/L) may have an increased lifetime risk of ASCVD similar to that of people with heterozygous familial hypercholesterolaemia (HeFH).

Nevertheless, a matter of debate still stands: Lp(a) seems to be a major independent risk factor for acute coronary syndrome only in individuals <45 years; although Lp(a) and LDL-C are independently associated with CVD risk, at LDL-C levels <100 mg/dL the risk associated with elevated Lp(a) is attenuated in primary prevention[4]; in patients with metabolic syndrome, Lp(a) is not associated with the incidence of vascular events.

Relative to the effects of LDL-C lowering therapy on Lp(a) levels, no clear effects have been described: compared with placebo statins significantly increase plasma Lp(a) levels (+11%),[5] or rather have a neutral effect. In this latter meta-analysis, both baseline and on-statin Lp(a) concentrations were associated with an almost linear increment in CVD, particularly in patients with Lp(a) concentrations ≥50 mg/dL.[6] Conversely, a dose-dependent increase in Lp(a) levels has been noted upon graded doses of atorvastatin, whilst an opposite effect is exerted by the proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors that reduce Lp(a) by a mean of 20%.[1] First evidence came from the LAPLACE (LDL-C Assessment with PCSK9 Monoclonal Antibody Inhibition Combined With Statin Therapy) study in which evolocumab given to hypercholesterolaemic patients, already at statins, reduced Lp(a) up to 32% after 12 weeks of treatment. When the analysis was restricted to subjects with an Lp(a) above the detection limit, evolocumab reduced Lp(a) by 36%, an effect consistent across age, sex, race, and diabetes mellitus.[7] The Lp(a) lowering driven by PCSK9 inhibitors was also evident in trials involving familial hypercholesterolaemia (FH) patients. In the ODYSSEY FH I and FH II studies enrolling HeFH patients, alirocumab lowered Lp(a) by 17.3% and by 20.3%, respectively.[8] Similar findings were described in the TAUSSIG (Trial Assessing Long-Term Use of PCSK9 Inhibition in Subjects with Genetic LDL Disorders) trial in which evolocumab resulted in an absolute Lp(a) lowering of 7.3% when given for 48 weeks to FH receiving ($n = 34$) or not apheresis ($n = 72$).[9] Interestingly, the addition of PCSK9 inhibitors to a background of niacin therapy leads to a further 15% reduction in Lp(a) beyond that achieved with niacin monotherapy (range: −20% and −30%).[10]

Table 1 Impact of evolocumab on major adverse cardiac events in the FOURIER trial according to Lp(a) stratification

	ARR	HR	NNT
Lp(a) < median	0.95%	0.93	105
Lp(a) > median	2.49%	0.77	40
Lp(a) < 120 nmol/L	1.41%	0.89	71
Lp(a) > 120 nmol/L	2.41%	0.75	41

Moving to the most recent outcomes trial with evolocumab, in the FOURIER (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk) study, median Lp(a) levels of 37 nmol/L (interquartile range = 13-165) were reduced by a median of 11 nmol/L (27%), being carriers of higher levels female or patients with a history of myocardial infarction (MI) or peripheral artery disease. Concerning the risk of experiencing MACE, patients with higher Lp(a) concentrations at baseline were those who derived a greater reduction in the risk of MACE. In particular, 25 nmol/L reduction in Lp(a) corresponded to a 15% decrement in the relative risk. Overall, evolocumab was superior to placebo in lowering MACE by 23% in patients with Lp(a) levels above the median compared to a 7% in those below the median, findings translated to a number-needed-to-treat (NNT)/3 years of 40 and 105, respectively. When patients were stratified for Lp(a) levels below or above 120 nmol/L (50 mg/dL), the NNTs over 3 years were 71 and 41, respectively (*Table 1*). Finally, patients who achieved levels of both LDL-C and Lp(a) below the median were those at lowest risk of subsequent MACE, i.e. −28%.[11] Of note, same trends were found in real-world cohorts that have reported mean Lp(a) reductions of about 20-25%.

Relative to alirocumab, pooled analysis of phase 3 trials, i.e. placebo-controlled (with concomitant statin), ezetimibe-controlled (with concomitant statin), and ezetimibe-controlled (no concomitant statin), reported that median changes in Lp(a) were −25.6% vs. −2.5% in placebo-controlled studies and −21.4% vs. zero changes in ezetimibe-controlled studies. The main result of this analysis was evident in the group with Lp(a) baseline levels ≥50 mg/dL; against a 25% decrement in Lp(a) there was a significant 38% reduction in MACE [hazard ratio (HR) 0.62; 95% confidence interval 0.39-0.97].[12] These effects were regardless of race and ethnicity with a mean reduction from baseline of −28.3% in White, −20.4% in African-American/Black, −25,7% in Hispanic/Latinos, and −28.4% in non-Hispanic/Latinos.[13]

The ODYSSEY OUTCOMES study was the first demonstrating a therapeutic effect linked to a reduction in Lp(a), a finding independent of LDL-C, i.e. changes in Lp(a) were associated with a reduction of MACE (HR 0.994 per 1 mg/dL decrement in Lp(a)).[14] Stratification for baseline quartiles of Lp(a) predicted the absolute changes in Lp(a) levels, i.e. −1.6 mg/dL [quartile 1 = <6.7 mg/mL), −4.8 mg/dL (quartile 2 = 6.7 to <21.2 mg/dL), −13.4 mg/dL (quartile 3 = 21.2 to <59.6 mg/dL), and −20.2 (quartile 4 = ≥59.6 mg/dL). Consequently, an absolute risk reduction

in MACE, non-fatal MI or CHD death, and CV death of 0.4% was found in quartile 1 (<6.7 mg/dL), 1.4% in quartile 2, 2.3% in quartile 3, and 2.1% in quartile 4. Conversely, there was no relationship between the reduction in Lp(a) and ischaemic stroke, and all-cause mortality. The relative risk reduction in MACE was 5% (quartile 1), 15% (quartile 2), 21% (quartile 3), and 17% (quartile 4).

The Lp(a)-lowering effect driven by PCSK9 inhibition is a feature associated also to inclisiran, a long-acting silencing RNA designed to target the 3′ UTR of the PCSK9 mRNA. Patients at high risk for CVD with elevated LDL-C given the maximal tolerated doses of inclisiran [single (200, 300, and 500 mg) or two-dose regimens (100, 200, or 300 mg on days 1 and 90)] had an Lp(a) trend to reduction with 80% of participants showing reduced Lp(a) levels at the end of the trial.[15] Lp(a) was not significantly lowered up to 18.2% from baseline in the single-dose regimen and up to 25.6% in the two-dose regimen.[15]

Beyond these clinical observations, it appears that Lp(a) reductions upon PCSK9 inhibitor treatment are achieved by two different mechanisms, i.e. (i) when given as monotherapy, evolocumab reduces the production rate of Lp(a), not the fractional catabolic rate (FCR), (ii) when administered in combination with atorvastatin the FCR of Lp(a) increases significantly, without alterations of the production rate. This most recent study differs somewhat from previous findings. Although, alirocumab-induced Lp(a) reduction is independent of apo(a) phenotypes, and the presence or absence of a small size apo(a), compared to placebo, the reduced plasma Lp(a) levels are apparently associated with a raised median increase of Lp(a) FCR (+24.6%, $P = 0.09$) with no changes in the production rate. The Lp(a) lowering activity mediated by PCSK9 antagonists may be related to the dramatic LDL-C reduction, thus taking away a possible competitor for the binding to the LDL receptor (LDL-R). However, specific studies aimed to address this mechanism did not clearly indicate an involvement of the LDL-R in Lp(a) uptake.[16] A further hypothesis is the direct binding between PCSK9 and Lp(a), an assumption valid only in the presence of high Lp(a) levels and not corroborated by others *in vitro* data. Moreover, it should be considered that only one out of 175 Lp(a) particles binds PCSK9, representing a very small percentage of total Lp(a) that is complexed.[17]

For patients with progressive ASCVD and high plasma Lp(a), a potentially valuable therapeutic option is lipoprotein apheresis (LA), which can be carried out by different methodologies and at different time intervals. LA is a treatment for severe FH patients that allows the intermittent extracorporeal removal of atherogenic apolipoprotein B-100 containing lipoproteins from the circulation. The effect of PCSK9 inhibition on the frequency of standard apheresis treatments has been tested in the ODYSSEY ESCAPE study. Sixty-two HeFH patients undergoing weekly or Q2W lipoprotein apheresis were assigned to receive alirocumab (150 mg) or placebo for 18 weeks. Although lipoprotein apheresis was discontinued in 63.4% of patients on alirocumab and the rate was at least halved in 92.7% of patients, any additive effect of alirocumab on top of LA on Lp(a) levels was not found. Results of 5 years of prospective follow-up confirm that lipoprotein apheresis has a lasting effect on prevention of CV events in patients with Lp(a) hyperlipoproteinaemia.

The possibility of an additional CV benefit by the combined treatments has been challenged by Stiekema *et al.*[18] Primary endpoint of the Anitschkow study was to evaluate changes in arterial inflammation by using ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) which is correlated with arterial macrophage content and predicts CV events. In patients with Lp(a) basal levels of 80 mg/dL, evolocumab did not reduce arterial wall inflammation in the presence of modest Lp(a) attenuation (−14%). The arterial benefit of LDL-C reduction may be blunted in patients with persistently elevated Lp(a). This observation may open a new dawn in CV prevention since so far in FH patients changes in arterial wall inflammation has been significantly correlated with LDL-C change, whereas no correlation have been demonstrated for Lp(a), suggesting that most of the wall inflammatory changes, as assessed by ^{18}F-FDG PET/CT, appeared to be LDL-C driven. In addition, Lp(a) may contribute to CVD risk being more atherogenic than LDL since it contains both the proatherogenic components of LDL and the oxidized phospholipids (OxPL) which are abundant in the apo(a) tail and are crucial mediators of the arterial wall inflammation.

The relationship between PCSK9 and Lp(a) has been finally confirmed in genetic studies in carriers of *PCSK9* loss-of-function (LOF) mutations. Among carriers of Y142X (*rs67608943*) and C679X (*rs28362286*) of the REGARDS study, median Lp(a) levels were 63.2 nmol/L in carriers vs. 80.4 nmol/L in non-carriers with oxPL-apoB levels of 3.4 nM vs. 4.1 nM, respectively. LOF were associated with a slight larger number of apo(a) KIV repeats, i.e. 24 vs. 23 nM, respectively.[19] Similar results were described in carriers of *PCSK9* R46L LOF mutation, i.e. Lp(a) levels were 10 mg/dL in non-carriers vs. 9 mg/dL in heterozygous and 8 in homozygous. The corresponding levels of LDL-C were 124 mg/dL, 104 mg/dL and 97 mg/dL, respectively.[20]

Although existing PCSK9 inhibitors outcome trials did not enroll patients with high Lp(a), their use in ASCVD patients with high plasma Lp(a) corresponded to a higher number of subjects discontinuing LA. All-in-all, a selected strategy to treat patients with markedly elevated Lp(a) with a specific antisense oligonucleotide against Lp(a) may start a new era in this field, so far paved with limited therapeutic options.

Conflict of interest: none declared.

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