Chapter 6

Lipoprotein(a) — A Hallmark in Atherosclerosis and Cardiovascular Diseases

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Additional information is available at the end of the chapter

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Abstract

Lipoprotein(a) [Lp(a)] consists of a low-density lipoprotein (LDL)-like core and apo(a), a large molecular weight glycoprotein. Apo(a) is highly homologous to plasminogen, yet in contrast exhibits a unique size polymorphism that is characterized by an increasing number of kringle-IV (K-IV) repeats. The number of K-IV repeats ranges from n = 2 to n = 40 or even higher. Apo(a) is synthesized almost exclusively in the liver and there is still some debate whether the assembly of Lp(a) from LDL and apo(a) occurs inside the liver cells or in the circulating blood. The plasma Lp(a) concentration is markedly skewed reaching from <1 mg/dl up to >200 mg/dl. The plasma concentration is >90% genetically determined and correlates negatively with the number of K-IV repeats. In the apo(a) promoter, there are numerous response elements for transcription factors and nuclear receptors that regulate apo(a) expression. The HNF4α binding sequence appears to be the most important one in that respect, yet further work needs to be done to unravel the key features of apo(a) biosynthesis under different conditions. Importantly, activation of FXR causes the dissociation of HNF4α α from its response element and in turn a significant downregulation of apo(a) transcription.

Undoubtedly, Lp(a) is one of the most atherogenic lipoproteins and recent large epidemiological studies document quite impressively that Lp(a) is an independent causal risk factor for coronary heart disease (CHD) and myocardial infarction. This fact led to the development of specific medications to reduce Lp(a) in patients with high plasma concentrations. Among the registered lipid-lowering drugs, only nicotinic acid has a consistently significant Lp(a)-lowering effect, and we recently succeeded in elucidating the mode of action of this drug. There are numerous medications in the pipeline for the treatment of hyper-Lp(a). Among those that are currently in clinical trials, CETP inhibitors, PCSK9 antibodies, MTP inhibitors as well as antisense oligonucleotides (ASO), such as the specific APO(a)$_{Rx}$® from ISIS, which is directed against apo(a)-mRNA and appears to be the most promising drug as it lowers Lp(a) levels by more than 90%.

Lp(a) emerged as an important screening parameter to assess coronary atherosclerosis risk. Its quantitation in the clinical laboratory was, for a long time, quite problematic

since commonly accepted reference materials and standardized analytical methods were lacking. However, newer commercial assays based on nephelometry or turbidimetry, or ELISA using monoclonal antibodies that recognize single epitopes in apo(a), warrant comparable interlaboratory results.

Keywords: Metabolism, Fibrinolysis, Reference values, Medication

1. Introduction

Lipoprotein(a) [Lp(a)] was uncovered in 1963, and its role in atherogenesis has been a matter of debate for many years. This was caused to a certain extent by the fact that the function of Lp(a) was—and still is—unknown. Also, there exists no specific therapy for reducing elevated blood levels of Lp(a). Lp(a) consists of an LDL-like core and a specific antigen, apo(a). Apo(a) exhibits a great homology to plasminogen. For this reason, it was long believed that Lp(a) may play a role in hemostasis and fibrinolysis. There are numerous publications dealing with the role of Lp(a) in hemostasis (reviewed in ref. [1]) providing evidence that the atherogenicity of Lp(a) in fact might be due to a certain extent to pathophysiological effects in fibrinolysis. These findings, however, appear to be of little relevance for practical considerations. Of much greater importance is the causal relationship of elevated plasma Lp(a) with the incidence of atherosclerosis, coronary heart diseases and stroke [2–4]. Of note, on the other hand, are the findings that plasma Lp(a) levels rise with age, i.e. that nonagenarians exhibit significantly higher Lp(a) plasma levels than younger generations [5].

2. Lp(a) metabolism

The protein part of Lp(a) consists of two main components, apoB-100 and apo(a) [6]. ApoB-100, the main component of LDL, is biosynthesized in the liver and LDL is the end-product of VLDL catabolism. Yet, LDL also appears to be synthesized directly and secreted from the liver. Liver LDL, however, displays a different composition from VLDL-derived LDL. Apo(a) consists of 11 unique "kringle-IV's" (K-IV) that are highly homologous to kringle-4 of plasminogen. In addition, apo(a) has a variable number of so-called repetitive K-IVs, which is one of the main puzzles in the immunochemical quantification of Lp(a) (see below). In addition to the presence of K-IVs, apo(a) possesses one kringle-V and a nonactive protease domain; further details on the structure of apo(a) may be found in ref. [7]. The exact mode of the assembly of Lp(a) from LDL and apo(a) might be irrelevant for Lp(a) quantifications, yet it has important implications for the development of Lp(a)-lowering drugs and the interpretation of their mode of action. Mixing recombinant apo(a) with LDL in the test tube and incubation for a few minutes leads to the formation of an intact Lp(a) particle that is indistinguishable from native Lp(a). This led to the assumption that the assembly of Lp(a) takes part outside the liver in circulating blood. Turnover studies carried out in the laboratory of H. Dieplinger (Innsbruck), on the other hand, revealed that the synthesis rate of protein components of Lp(a), i.e. apoB-100 and apo(a), are

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identical but distinct from the synthesis rate of apoB-100 in LDL [8]. This appears to be a strong argument for the intracellular assembly of Lp(a).

It is well established that the black population has strikingly different plasma Lp(a) concentrations compared with the white population (black individuals have the highest and Asians have the lowest plasma Lp(a) levels [9]). In addition to these ethnic differences, there are great differences of plasma Lp(a) levels in any ethnic group ranging from <1 mg/dl to >200 mg/dl. This heterogeneity is caused, on one hand, by numerous polymorphisms in the apo(a) promoter and on the other hand by the variable number of K-IV repeats: individuals with a high copy number of K-IV have lower plasma Lp(a) levels and those with a low number copy number have higher plasma Lp(a) levels.

3. The biosynthesis of apo(a)

The locus for the apo(a) gene is situated at chromosome 6 (6q26–q276). The biosynthesis of apo(a) is characteristic of that for any glycoprotein, and the negative correlation of the number of K-IV repeats with the plasma concentration has been explained by longer cellular residence times causing a more efficient intracellular degradation of large molecular weight apo(a) isoforms.

The rate of apo(a) biosynthesis, on the other hand, is significantly influenced by the promoter activity and its activation by transcription factors and nuclear receptors. We provided evidence that the apo(a) promoter contains response elements for >70 transcription factors including HNFs, FXR, PPARs, RXR, SREBPs, CCAAT-Enhancer, IL-6 in addition to numerous others that play important roles in the lipid and lipoprotein metabolism [10]. The presence of these multiple transcription factor binding sites led to the assumption that the regulation of apo(a) transcription might be complex and influenced by numerous metabolic features. Our research group was in fact the first to characterize important response elements in the apo(a) promoter that are key for apo(a) transcription and the abundance of apo(a) in blood plasma [10]. The most significant response element is that of HNF4α at position –826 to –814 in the apo(a) promoter. HNF4α $\beta\nu\delta\nu\gamma$ is competed by farnesoid-X receptor (FXR), a nuclear receptor that plays an important role in bile acid metabolism. Thus, elevated plasma bile acid levels that activate FXR lead to a profound reduction of Lp(a) biosynthesis. These metabolic relationships are schematically displayed in Fig. 1.

4. The Lp(a) catabolism

There exist numerous gaps not only in the understanding of Lp(a) biosynthesis but even more so in Lp(a) catabolism. Very little is known on the site of uptake, the mode of cell binding, internalization and intracellular degradation. *In vivo* studies have been performed mostly on animals that by themselves do not produce Lp(a). These latter studies revealed that >50% of Lp(a) is taken up by the liver and that protein degradation products are secreted into bile [11].

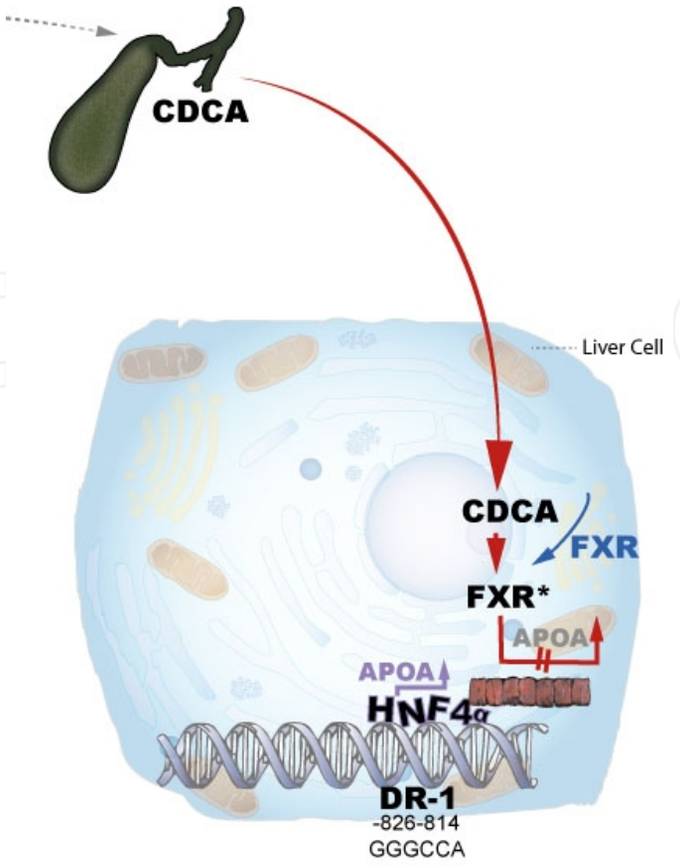

Figure 1. Inhibition of apo(a) transcription by bile acids. Chenodeoxycholic acid (CDCA), the FXR agonist with the highest affinity in humans, binds and activates FXR leading to a displacement of that complex from the cytoplasm to the nucleus. The complex interferes with HNF4α binding to DR-1, a key response element in the apo(a) promoter and in turn silences apo(a) transcription. With permission of the Medical University of Graz (copyrights held by the MUG Graz, Austria).

Significant amounts of radioactivity from labeled Lp(a), however, were also found in kidney, spleen, lung and pancreas, yet it is unknown whether these organs are of relevance for Lp(a) catabolism in humans. Since the liver is the principal organ for the LDL-receptor-mediated catabolism of apoB-containing lipoproteins, it was of interest to study this particular pathway for Lp(a) catabolism. *In vivo* studies carried out in our laboratory as well as by other groups, however, revealed that Lp(a) only has a low affinity to the LDL-R. The main argument for this allegation is the fact that Lp(a) is catabolized in homozygous FH patients with the same rate as compared to healthy control individuals [12]. Since pathways involved in Lp(a) catabolism —and in particular the role of specific receptors—is of eminent importance for strategies to develop Lp(a)-lowering drugs, many attempts have been made to identify binding proteins (receptors) that might be specific for Lp(a). Actually there is hardly any lipoprotein receptor that had not been found to bind Lp(a), including LRP, VLDL-R, asialo-glycoprotein receptor, different scavenger receptors and others. Unfortunately, all these results are based on *in vitro* studies that may have little relevance for the *in vivo* situation. One pathway that appears to be a hot candidate for Lp(a) catabolism is the binding of apo(a) kringle to lysine (Lys)-rich cell surface proteins. Along these lines, we actually demonstrated in previous experiments that

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feeding of Lys analogues such as tranexamic acid or δ-amino valeric acid to transgenic apo(a) or Lp(a) mice increased the Lp(a) plasma levels by a factor of 1.5–2, and this correlated with a lower cell uptake and a higher Lp(a) degradation [13].

5. Lp(a): A causal risk factor for atherosclerosis, CHD and stroke

In MedLine and other databanks, there are more than 1500 papers listed dealing with this topic. Thus, it is almost impossible to consider all these publications in this report. Therefore, in this article, we concentrate mainly on the most recent findings; this does not imply that older references might be of lesser relevance. Semiquantitative measurements of Lp(a) in a Scandinavian collective by the "father" of Lp(a), Kare Berg, revealed that individuals with a more pronounced "sinking pre-β band" [= Lp(a)] is found in lipoprotein electrophoresis correlated with the appearance of angina pectoris and CHD [14]. The first quantitative measurements of Lp(a) were in fact carried out by rocket electrophoresis in our laboratory in cooperation with P. Avogaro from Venice. In that case-control study, where 183 probands were included, it was found that the relative risk (RR) of suffering from myocardial infarction (MI)—depending on the applied cutoff value—was approximately 2-fold higher than in healthy controls [15]. This led to the adoption of an upper cutoff value of 30 mg/dl in most subsequent studies. In our first publication, we also could show that patients with type-IIa hyperlipoproteinemia (familial hypercholesterolemia, FH) exhibited a 6-fold higher risk of myocardial infarction (MI). Most of the subsequent studies that were published from various laboratories confirmed a positive correlation of Lp(a) plasma levels with CHD and MI. Some of the studies in fact were also negative, i.e. no relation of Lp(a) with atherosclerotic diseases could be established (for a review, see ref. [16]). A stab in the back to Lp(a) research in fact was given in 1993 by the article from Ridker et al. [17], who could not find any significant relation between Lp(a) and the risk for CHD in a nested case-control evaluation in the Physician's Health Study with almost 15,000 probands "In this prospective study of predominantly middle-aged white men, we found no evidence of association between Lp(a) level and risk of future MI. These data do not support the use of Lp(a) level as a screening tool to define cardiovascular risk among this population." These findings of Ridker et al [17] might have been based on the fact that the methodology used for Lp(a) quantification was subject to criticism.

Some years later, Lp(a) research encountered a revival after the publication of new data from several research groups in 2009–2011. These studies comprised >100.000 patients or probands and, for the first time, revealed beyond any doubt a significant causal relationship between elevated plasma Lp(a) and CHD ([2–4, 18,19]). Of note are studies from the last 3 years which underline the significance of Lp(a) as a risk factor for atherosclerotic cardiovascular diseases:

The PROCARDIS Consortium asked the question that had been discussed for a long time, whether different apo(a) isoforms with different number of K-IV repeats would exert differences in their atherogenicity [20]. There were actually indications in the literature that not only the actual plasma concentration of Lp(a) but also the size polymorphism may reflect the risk of atherosclerosis. Thus, in the PROCARDIS study, including some 1000 patients and a similar

number of control individuals, plasma Lp(a) concentrations were measured by latex-enhanced immune-turbidimetry (see below) and apo(a) isoforms were assayed by SDS-polyacrylamide gel electrophoresis followed by immune blotting, using the isoform-standard from Immuno A.G., Vienna. Unfortunately, Immuno A.G. does not exist anymore and isoform standards are nowadays hard to obtain. The authors of PROCARDIS calculated the odds ratio (OR) of patients and controls between the first and last quintile before and after adjusting for the number of K-IV repeats. In both calculations, an OR of 2.05 ($p < 0.001$) was found, i.e. no difference could be observed whether the apo(a) size polymorphism was taken into consideration or not. This report appears to quite definitely conclude this debate and is proof that Lp(a) exerts its atherogenicity through its plasma concentration and not through possible structural differences in K-IV repeats. In an editorial to this report, F. Kronenberg (Innsbruck) pointed out that the analysis of SNPs—in particular rs41272114, rs10455872 and rs3798220, which exhibit the strongest association to plasma Lp(a) concentrations can neither be taken as surrogates nor as substitutions for the number of K-IV repeats. He further pointed out that more than half the number of individuals with isoforms containing less than 22 K-IV repeats are not recorded by this SNP analysis mentioned above.

In a further publication by the PROCARDIS Consortium published in ATVB [21], the question was asked as to what extent the LPA "null allele" (rs41272114) might influence the plasma concentration of Lp(a) in heterozygous individuals and if it might be a determinant for atherogenic risk. In this study comprising some 8000 CAD patients, an allele frequency for rs41272114 of approximately 3% was found. Patients containing the null allele exhibited significantly lower plasma Lp(a) levels as compared to control individuals without the rs41272114 allele (OR 0.79; $p = 0.023$). According to findings from the group of G. Utermann [22], the rs41272114 SNP represents a donor-splice site mutation leading to the biosynthesis of a truncated apo(a) with only 7 K-IVs (K-IV 1–7) in total and no K-V or protease domain. As a consequence of the absence of K-IV type 9, which contains the only free –SH group in apo(a) and is responsible for the covalent binding to apoB-100, the truncated apo(a) fragments are well secreted from the liver into the blood but do not assemble with LDL and thus are rapidly degraded and removed from the circulation. The PROCARDIS study also proved that individuals with only one apo(a) isoform exhibit a large variation in their plasma Lp(a) concentrations and that there exists a sigmoid correlation between the number of K-IV repeats and plasma Lp(a) levels. The question of the mechanism that causes this variation, however, could not be answered by this study. The authors of the PROCARDIS Consortium claimed, on the basis of their results, that in future epidemiological studies by SNP analysis for the assessment of the CAD risk, the rs41272114 polymorphism must be taken into consideration as a matter of state-of-the-art experiments.

Further support of the hypothesis published in 1981 by our group [15] indicating that Lp(a) might be a significant risk factor for MI comes from the "Bruneck Study" comprising 826 male and female probands [23]. In a recall survey after 15 years, it was found that the inclusion of Lp(a) in the Framingham algorithm for the risk assessment of CHD, an improvement of 0.016 in the C-index was reached. Consideration of Lp(a) plasma levels improved the hit rate in the prediction of CHD by 40%.

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6. Lp(a) and stroke

The question as to what extent Lp(a) might also be causally related to stroke was addressed in numerous publications (reviewed in ref. [24]). Sultan et al. [25] recently published the results of his meta-analysis, where he included 10 published papers dealing with ischemic stroke in children. Setting the cutoff level for Lp(a) at 30 mg/dl, a positive association between Lp(a) and stroke was found with a Mantel-Haenszel OR of 4.24 ($p < 0.00001$).

As mentioned above, the physiological function of Lp(a) is in the dark. Concerning the pathophysiology, the work of Tsimikas et al. from San Diego is noteworthy because they believe that the high affinity of Lp(a) for oxidized phospholipids might be responsible for its atherogenicity [26]. Oxidized phospholipids are known to promote the synthesis of inflammatory cytokines that recruit monocytes and T-lymphocytes. Monocytes differentiate to macrophages that phagocytose oxLDL and are transformed to foam cells, hallmarks in atherogenesis. Negatively charged phospholipids such as Ox-Phos are key components in oxLDL and also bind a specific protein, β-2-glykoprotein-I (β2-GPI). The latter also forms a complex with Lp(a). In a recently published paper, it was reported that the plasma levels of Lp(a), Ox-Lp(a) and β2GP-I-Lp(a) in stroke patients were significantly higher than in controls (124 patients vs. 64 controls). In addition, a positive correlation of these plasma parameters with the severity of stroke was established [27]. These findings point towards the assumption that Lp(a) might not neutralize ox-PL but in contrast boosts its atherogenic properties.

7. Medication of patients with elevated plasma Lp(a)

Although recent publications might be taken as a clear hint for the causal relationship between elevated Lp(a) and CHD, a final proof might be only obtained from intervention studies with Lp(a)-lowering drugs (see ref. [28]). Unfortunately, there hardly exists any drug that may specifically lower plasma Lp(a). As a consequence of the work cited above, however, intensive efforts have been taken to develop such therapeutic agents. In addition, any new lipid-lowering or HDL-raising drug that is in clinical trials is checked for its potential action on Lp(a). There exist several serious recommendations for the treatment of relevant patients (reviewed in ref. [16]), but many of them are based on anecdotal observations, or are of little practical value or low efficacy. As mentioned above, Lp(a) is hardly bound by the LDL-R and thus specific drugs acting solely by increasing LDL-R activity are mostly inactive in the treatment of hyper-Lp(a) patients. Statins that belong to this sort of drugs are therefore not the drugs of choice—and actually there are reports that statins even may lead to an elevation of Lp(a) [29]. Unfortunately, the pathomechanism of the LDL-raising effect of this observation is unknown.

The only current method that reduces plasma Lp(a) levels to a satisfactory extent is apheresis, and it has been shown that lowering Lp(a) by this method reduced the CHD risk significantly [30]. Apheresis is therefore strongly advised in the secondary prevention of patients with very high plasma Lp(a) (>80 mg/dl).

Another current possibility is medication with nicotinic acid or derivatives thereof. Nicotinic acid (niacin), its amide or different retard compounds were, for a long time, in the markets of numerous countries because of their HDL-raising properties. In addition, these compounds reportedly reduce plasma Lp(a) by some 30% [31]. In recent studies, we succeeded to uncover the mode of action of this drug at a molecular level: the *APOA* promoter contains several cAMP response elements that impact the apo(a) transcription [32]. Nicotinic acid interferes in liver with the binding activity of cAMP to these elements and reduces the biosynthesis of apo(a) (see cartoon in Fig. 2).

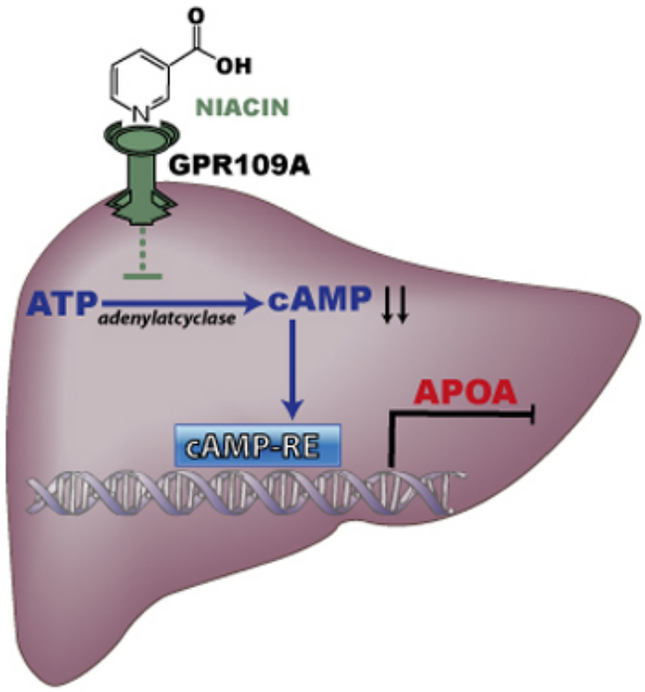

Figure 2. Influence of nicotinic acid on apo(a) biosynthesis: Proposed mode of action. cAMP regulates apo(a) biosynthesis by binding to specific cAMP response elements in the *APOA* promoter. Nicotinic acid inhibits adenylate cyclase, the key enzyme of cAMP biosynthesis in the liver and in turn lowers its intracellular concentration, leading to a lower expression of the *APOA* gene. With permission of the Medical University of Graz (copyrights held by the MUG Graz, Austria).

A major side effect of nicotinic acid is the activation of prostaglandin D_2, particularly in the skin, which causes the dilatation of blood vessels by binding to DP1 (PGD2 receptors) and in turn causing skin flushing (red face). Thus, nicotinic acid is not very well appreciated by most patients and, as a consequence, it was removed from the market in most countries. Another drawback for nicotinic acid was the outcome of the HPS2-THRIVE study (http://www.nejm.org/doi/full/10.1056/NEJMoa1300955). In this trial, 25,673 patients were treated with a standard statin background therapy plus a nicotinic acid supplement consisting of 2 g extended-release niacin + 400 mg of the DP1 antagonist laropiprant or a matching placebo. As it turned out, the supplement nicotinic acid-laropiprant therapy did not reduce CHD risk but increased the incidence of serious adverse events.

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8. Selective medication for hyper-Lp(a)

In our study cited in ref. [10], we actually found that patients suffering from extrahepatic cholestasis exhibited very low Lp(a) plasma concentrations. After treating these patients and curing them from cholestasis, Lp(a) levels vent up significantly. In this study, we succeeded in pinpointing FXR as the most important repressor for apo(a) biosynthesis (see also Fig. 2). Unfortunately, FXR is a pluripotent nuclear receptor that plays eminent roles not only in bile acid and glucose metabolism but also influences the activity of LXR, the master regulator of cellular cholesterol metabolism. There exist negative feedback loops not only between FXR and LXR but also between FXR and other transcription factors, cytokines and interleukins. Thus, the application of FXR activation must be done with great caution and may be not feasible at all for prolonged applications. Nevertheless, such FXR agonists are in development and are currently being tested for their action on the plasma levels of Lp(a). Phenex, a SME that specializes on the development of antagonists and agonists of nuclear receptors, has the FXR agonist Px-102® in clinical trials (http://www.phenex-pharma.com/pdf/PR-Phenex-Phase%20I%20finished_5%20M%20Euros_engl.pdf). Px-102® significantly affects plasma cholesterol levels in laboratory animals and is also tested for its potential effect on liver tumors. No data has been released so far on its potential effect on Lp(a).

Other selective Lp(a)-lowering agents are currently under investigation. They comprise mostly drugs that were originally drafted to lower LDL cholesterol or increase HDL cholesterol. Among them, inhibitors of PCSK-9, CETP, MTP and thyromimetica are worth noting.

8.1. PCSK-9 inhibitors

Recently, it has been published in *Circulation* that AMG145, the monoclonal antibody against PCSK-9 from Amgen®, at a dose of 105 mg Q2W reduced plasma Lp(a) levels on average by 32% [33]. At this dose, the authors observed a reduction of plasma LDL-C and of apoB by 60% and 50%, respectively. It must be stressed, however, that among the 626 male and female patients, approximately half of them had Lp(a) levels below the median concentration of 43 nmol/L. Although the Lp(a)-lowering effect of AMG-145 correlated significantly with the reduction of LDL-C, patients with low Lp(a) showed a much bigger relative reduction of Lp(a) than patients of the 3rd or 4th Lp(a) quartile. At a dosage of 420 mg, Q4W patients of the 4th quartile did not respond at all with a reduction of Lp(a). According to unconfirmed communications AMG-145 might be registered in the 2nd half of 2015, yet the treatment costs will certainly be significantly higher as compared to that of statin therapy.

In a similar study with SAR236553, the PCSK-9 antibody from Sanofi®, an average reduction of Lp(a) of up to 28.6% was observed [34].We actually consider these trials as pilot studies as they do not address all the questions of the mode of action of PCSK-9 inhibitors on Lp(a). It is well known that these drugs increase the activity of LDL-R, particularly in the liver, and this receptor has a relatively low affinity to Lp(a).

8.2. CETP inhibitors

CETP stands for "cholesterol ester exchange/transfer protein." It catalyzes the exchange of CE and triglycerides between VLDL or LDL and HDL. In fact, many years ago, we published that Lp(a) also serves as a substrate for CETP [35]. On theoretical grounds, CETP inhibitors should be ideal for the treatment of stroke patients that exhibit significantly elevated Lp(a) and reduced HDL levels [36]. The development of drugs containing the CETP inhibitors Torcetrapib and Dalcetrapib has been stopped because of unwanted side effects. Anacetrapib and Evacetrapib, on the other hand, are currently in phase II clinical trials. Concerning Anacetrapib, it was published in several reports that it reduces Lp(a) by up to 25%, yet details of this study are still lacking.

Further medications such as Eprotirome, a thyromimetikum, Lomitapide, an MTP inhibitor from Aegerion, and Mipomersen, an antisense oligonucleotide targeting apoB, all reportedly reduce Lp(a); however, it is rather uncertain that these drugs will ever be admitted for the indication, high Lp(a).

The most promising medication, at the time being, appears to be $APO(a)_{rx}$, a specific antisense drug from ISIS®. In that respect, it is noteworthy that we published in 2001 that by RNA interference, a 100% inhibition of the expression of ap(a) may be accomplished in transgene apo(a) mice [39]. ISIS®, in fact, claims from a phase I study that in patients with Lp(a) of 10–100 mg/dl, a reduction of up to 90% was reached (http://www.isispharm.com/Pipeline/Therapeutic-Areas/Cardiovascular.htm#ISIS-APOARx). If ISIS ® succeeds in admitting their antisense drug $APO(a)_{rx}$, we consider this strategy as the most specific and effective in treating hyper-Lp(a).

9. How and when should Lp(a) be measured

Actually, Lp(a) is not measured routinely in clinical laboratories because of the following reasons: (i) there exists no effective treatment regime to lower plasma Lp(a); (ii) the currently available Lp(a) assays are not standardized and results from different laboratories vary considerably (see ref. [16]).

i. As mentioned above, very effective and partially specific Lp(a) medications are in clinical trials and it is hoped that some of them might soon be on the market. Even if it should take several years before such drugs are admitted, knowledge of the plasma Lp(a) value gives additional important information to judge CHD risk. It has been reported previously that plasma Lp(a) levels stay pretty constant over months and years and may hardly be influenced by diet and living conditions. Systematic studies within single individuals, however, revealed quite large fluctuations. Patients with elevated or borderline Lp(a) values therefore should be assayed for Lp(a) at several occasions.

ii. It is true that most commercial Lp(a) assays are not standardized, and the accuracy and precision of these assays needs to be seriously revised. Since there is a strong

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genetic component in Lp(a) plasma levels, Lp(a) should also be measured in all relatives of such index patients mentioned above. In addition, we advise that Lp(a) should be measured in the plasma of all premature myocardial infarction and stroke patients in addition to patients with borderline CHD risk because elevated Lp(a) puts them into a higher risk group. Since a specific practicable Lp(a) therapy is currently lacking, patients at increased CHD risk that exhibit elevated Lp(a) must be treated quite more rigorously with conventional lipid-lowering therapy than similar patients with low Lp(a). The monitoring of Lp(a), in addition, is advised in patients that undergo state-of-the-art therapy but still show a progression of atherosclerosis or vascular diseases, in all FH patients and in patients with genetic lipoprotein disorders, in patients with low HDL or high homocysteine, or in patients with disorders of hemostasis or fibrinolysis. Finally, we recommend assaying Lp(a) in patients with diabetes mellitus and autoimmune diseases.

In a consensus report of the European Atherosclerosis Society, monitoring of plasma Lp(a) is recommended in patients at a 10-year atherosclerosis risk of >3%. Particular attention should be paid to hemodialysis patients and patients with any form of kidney disease. In kidney disease, it is important before targeting hyperlipoproteinemias by medication to treat the primary disease as well as possible and to concentrate on modifiable CHD risk factors such as LDL-C, high blood pressure, smoking, blood glucose and obesity. Apheresis in addition to nicotinic acid therapy must be considered in these patients if feasible, although evidence-based results are still lacking.

10. What needs to be kept in mind when measuring Lp(a)

The first laboratory methods for measuring plasma Lp(a) were radial immune diffusion, rocket electrophoresis and later nephelometry. Today, high-throughput methods are mostly requested comprising ELISE, DELFIA nephelometry and turbidimetry. In all these methods, one must consider the fact that the molecular mass of Lp(a) and apo(a) varies quite strikingly within large limits, that Lp(a) contains apoB-100 in addition to apo(a), that Lp(a) exhibits great affinities to other proteins, e.g. β2-GPI, and in particular that apo(a) contains repetitive structures: the number of repetitive K-IV repeats varies from 2 to approximately 40 or more. This causes, in many assays, an overestimation of Lp(a) concentrations in patients with large apo(a) isoforms and an underestimation in patients with small apo(a) isoforms. Finally, one must consider the presence of small apo(a) fragments in plasma that are not bound to LDL. Yet the concentration of these fragments correlates positively with Lp(a) levels. In order to circumvent some problems in the quantitative analysis of Lp(a), ELISA and DELFIA methods have been suggested where the capture antibody binds relatively unspecific all apo(a) isoforms yet the detection antibody is monoclonal and recognizes only one epitope in apo(a). Other assays use anti-apoB as a detection antibody. This, however, is biased by the fact that in hyperlipidemic samples, one Lp(a) particle may bind additional apoB-containing lipoproteins leading to an overestimation of plasma Lp(a) levels.

As a consequence of these challenges, a group of experts in the field vent together that tackled these problems by propagating various reference standards and methods. We also participated in this survey using our in-house laboratory methods and antibodies [40]. A major problem that came out from this study was the different reference materials used in the assays. Even the use of the WHO/IFCC Reference Material as a common calibrator did not result in satisfactory harmonization of Lp(a) values [41].

We consider most of the considerations, based on theoretical grounds, of little importance in commercial routine clinical assays. There are three important questions that need to be solved: (1) What methods are apo(a)-isoform insensitive? (2) How can be units in mg/dl be transformed into nmol/L? (3) What are the cutoff levels to be adopted for risk stratification?

11. What needs to be considered in measuring Lp(a)?

Considering all these puzzles, we propagate the following: Our preferred commercial assays are based on latex-enhanced immune-nephelometry or -turbidimetry. This is based on the consideration that the size of latex particles in comparison to the size of Lp(a) is very high and the size polymorphism of apo(a) becomes negligible. In addition, the latter methods are highly precise and may be applied in high-throughput. ELISA and DELFIA methods may be isoform-insensitive if monoclonal antibodies are used that recognize only one epitope on apo(a).

Another possibility that still needs confirmation is to assay apo(a) fragments in urine. In urine, apo(a) fragments are secreted by a mechanism that has not been fully explored so far. These fragments consist mostly of repetitive K-IV structures of different lengths and thus what is measured accurately even by the use of polyclonal antibodies is the concentration of K-IVs, mostly of type 2, that have been shown to correlate significantly with the plasma Lp(a) concentration [42]. In the work cited in ref. [42], we could actually show that the discriminatory power of urinary apo(a) fragments is at least as good—if not even better—than that of plasma Lp(a).

12. Should Lp(a) levels be expressed as mass units or molar units?

This question is partly academic since there is, at the moment, no validated commercial assay on the market that gives accurate and reliable molar values. One must also consider that most of the individuals are heterozygous, i.e. they have 2 kinds of Lp(a) particles in their blood with quite large differences in the molecular mass. In our laboratory, we use mass values since the majority of the published epidemiological studies publish their values in mg/dl or mg/L. In addition, the cutoff values propagated in the Consensus Report of the European Atherosclerosis Society are given in mg/dl.

Keeping in mind that not only the molecular mass but also the composition of Lp(a) varies quite remarkably, we must think practicable for the time being. Assuming a molecular mass

for Lp(a) of 3,150.000 Daltons, a value that sounds quite realistic on the basis of quasielastic light-scattering data, a conversion factor of 3.17 for converting mass into molar units has been proposed: 1 mg/dl apo(a) corresponds roughly to 3.17 nmol/L. It should be pointed out, however, that in the US, a conversion factor of 2.5 has been proposed. This factor may be calculated on the grounds of a molecular mass of 4 million.

What are the most realistic cutoff values? Most of the results from recent studies assumed that Lp(a) is not a continuous risk factor but rather that a significant risk starts at a certain border value. This in fact is not supported by any evidence-based study, yet on practical considerations, cutoff levels have been propagated. In the original study where Lp(a) was quantitatively measured in our laboratory, we published that at a cutoff point of 30 mg/dl, the relative risk for myocardial infarction in that particular collective was 1.75 and at a cutoff value of 50 mg/dl, the relative risk was 2.5 [15]. These values are very close to those that have been obtained in numerous large epidemiological studies including meta-analyses of prospective trials published by many laboratories. The European Atherosclerosis Society propagates, in a consensus report that is mostly based on data from the Copenhagen Heart Study, a cutoff value of 50 mg/dl, corresponding to approximately 150 nmol/L.

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