Lp(a) Paradox in Diabetes Mellitus

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Lipoprotein(a) (Lp(a)) is one of the strongest causal risk factors of atherosclerotic disease. It is rich in cholesteryl ester and composed of apolipoprotein B and apo(a). Plasma Lp(a) levels are determined by apo(a) transcriptional activity driven by a direct repeat (DR) response element in the apo(a) promoter under the control of (HNF)4α Farnesoid-X receptor (FXR) ligands play a key role in the downregulation of APOA expression. In vitro studies on the catabolism of Lp(a) have revealed that Lp(a) binds to several specific lipoprotein receptors; however, their in vivo role remains elusive. In patients suffering from Type-I diabetes mellitus (T1DM), provided they are metabolically well-controlled, Lp(a) plasma concentrations are directly comparable to healthy individuals. In contrast, there exists a paradox in T2DM patients, as many of these patients have reduced Lp(a) levels; however, they are still at an increased cardiovascular risk. The Lp(a) lowering mechanism observed in T2DM patients is most probably caused by mutations in the mature-onset diabetes of the young (MODY) gene and possibly other polymorphisms in key transcription factors of the apolipoprotein (a) gene (APOA).

Keywords: lipoprotein(a) ; diabetes mellitus ; type-1 DM ; type-2 DM ; metabolism

1. Lp(a) Structure

In different types of electrophoresis, Lp(a) is displayed as a lipid stainable, distinct band in the pre-β1 region between β- and pre-β (LDL and VLDL) lipoproteins. With ultracentrifugation, most Lp(a) is found in the HDL_1 region, although, depending on the isoform, Lp(a) may also hide in the LDL or HDL2 fractions. Additionally, in the plasma of heterozygote individuals with two distinct polymorphic apo(a) forms, two Lp(a) bands are frequently observed by density gradient ultracentrifugation. It is important to note that lipoproteins are purified mainly from the fasting plasma of healthy individuals. Under these conditions, an idealized Lp(a) particle is mostly found to consist of an LDL core particle surrounded by one apo(a) glycoprotein. Similar pictures can be found in numerous articles on Lp(a).

In normolipemic fasting plasma, approximately 75% of the immune reactivity is found in the HDL_1 region, while the rest distributes amongst VLDL, LDL, whole HDL, and the bottom fraction. In non-fasting plasma, the distribution of apo(a) is even more heterogeneous. This is particularly true in plasma with elevated triglyceride-rich lipoproteins (TGRLp), as observed in patients with T2DM. The exact structural features of Lp(a) found outside the HDL fraction have not been fully explored. However, unpublished work suggests that TGRLp contains apo(a) that is not fully assembled with apoB-100, in addition to some Lp(a) aggregates. Apo(a)-containing fractions isolated by immune-adsorbers consist partly of fractions with apoB:apo(a) ratios >1 or, in other words, LDL:Lp(a) complexes. Although the apo(a) found in the lipid-free bottom fraction after ultracentrifugation may contain small amounts of full-length apo(a), most of it consists of apo(a) fragments created by proteolytic enzymes [1].

Extensively purified Lp(a), isolated from the fasting plasma of healthy donors by several consecutive purification steps, consists of one LDL core particle (that is indistinguishable from LDL of density 1.016–1.063) and one apo(a) glycoprotein, linked together by one disulfide bridge.

Apo(a), the specific antigen of Lp(a), has a very characteristic structure and shares close homology to plasminogen (Plg) [2][3]. In addition to a protease domain, Plg has five distinct kringle sections, numbered I-V. Apo(a) cDNA is 75–100% homologous to Plg, with a variable number of kringle-IV (K-IV), one K-V-like domain, and a protease-like domain. The numerous K-IVs in apo(a) are only partly identical; in fact, ten different subtypes of K-IVs have been found. Where K-IV-1 and K-IV-3—K-IV-10 (the so-called “unique kringles”) are present only once, a variable number of K-IV-2 exist amongst different individuals. This is the characteristic feature responsible for the size heterogeneity of apo(a) observed in different individuals, where up to 50 K-IVs have been identified.

There is an urgent dispute in the literature regarding the units of measurement for Lp(a). In most publications up to approximately 2015, Lp(a) was expressed in mg/dL, and a cut-off between 30–40 mg/dL was assumed for CVD. Given

that the composition of Lp(a) is extremely variable, it was concluded that concentration units should only be expressed in nmol/L. While this is indisputably correct, most high throughput Lp(a) assays are based on immune turbidimetric or nephelometric methods using polyclonal antibodies. Due to the variable number of K-IV repeats, such methods overestimate the concentration of large isoforms and underestimate that of small isoforms. Therefore, it is not a straightforward method to apply one conversion factor of mg/dL into nmol/L for plasma samples with Lp(a) of different isoforms. An additional problem may be that apo(a) is a glycoprotein with a carbohydrate content of approximately 28%, and this is mostly neglected when calculating the true molecular mass.

The theoretical molecular mass of apo(a) with 20 K-IV repeats, including the carbohydrate moiety, is 368,016.26 Daltons (D). For each additional K-IV, 20,361 D must be applied. The molecular mass of the core LDL is variable, yet an average value of 2.8 million D is propagated in the literature. Thus, the mass of Lp(a) with 21 K-IV repeats is roughly 3.17 million D. On the basis of this value, a theoretical conversion factor of 3.15 (1 mg/dL = 3.15 nmol/L) may be calculated.

Recently, the theoretical mass of Lp(a) with 6 to 35 K-IV-2 repeats was calculated by Cobbaert and Ruhaak [4] to range from 2821 to 3344 kD. At the basis of these values, the calculated conversion factors are 3.54–2.99. It must be stated here that manufacturers of Lp(a) assays propagate much lower conversion factors between 2.2–2.4. Therefore, it is evident that further research in this area is crucial in order to determine fixed widely acceptable Lp(a) units.

2. Lp(a) Metabolism

2.1. Biosynthesis and Assembly

Apo(a) is biosynthesized almost exclusively in the liver, yet small amounts of apo(a) mRNA have also been identified in the brain and testis [2]. The significance of these two latter organs in Lp(a) metabolism remains obscure.

In early investigations, scholars studied the turnover of Lp(a) in healthy individuals and demonstrated for the first time that the plasma Lp(a) concentration highly and significantly correlates with the rate of biosynthesis. However, no correlation could be found with the fractional catabolic rate (FCR) [5].

In comparison, in individuals with elevated LDL-C, the FCR of Lp(a) correlated significantly with that of LDL.

Although the reason for this observation was not fully explored, it is tempting to assume that the rate of LDL biosynthesis might be responsible for these observations. Furthermore, the plasma Lp(a) concentration may also be driven by the speed of LDL production. The observations have been confirmed in numerous subsequent studies and are most relevant in strategies for pharmacological interventions in patients with hyper-Lp(a): Drugs for lowering plasma Lp(a) must reduce apo(a) biosynthesis and/or the Lp(a) assembly, whereas naturally increasing its catabolism will most likely fail.

The expression of the *APOA* gene follows general principles of transcription → translation → post-translational modifications and secretion from cells. For gene transcription, positive and negative regulatory elements are key. Scholars addressed this question and identified >70 response elements in the apo(a) promoter for transcription factors and nuclear receptors. The significance of apo(a) expression in most of them is still unknown, yet the most important one could be characterized by promoter expression studies [6].

After transcription and translation, apo(a) is heavily –*N* and –*O* glycosylated and passes the Golgi apparatus ready for secretion. As mentioned above, under normal conditions, >95% of apo(a) found in plasma is bound to genuine LDL that is sparsely found in the liver but rather derives from TG hydrolysis of VLDL in circulating blood. Thus, the question arises where and how the assembly of native Lp(a) occurs. Early research revealed that a genuine Lp(a) might be synthesized in vitro in the test tube by mixing purified LDL with recombinant apo(a) in the absence of any cofactor. It was, therefore, speculated that in vivo, apo(a) gets secreted from the liver and binds in a similar way to apo(a) by the interference of lysine groups on apoB-100 with specific kringle domains in apo(a). For this first step, K-IV-3 and K-IV-6 appear to be most relevant. In a second step, the apo(a):apoB-100 complex is stabilized by a disulfide bridge formed between Cys-3000 in apoB and the only free lysine group in K-IV-9. Early work published by White and Lanford [7], however, only partially supported this hypothesis, as they demonstrated by using baboon liver cell cultures, that apo(a) during secretion is bound to the cell surface. Upon contact with mature LDL, these two proteins associate and form a genuine Lp(a).

2.2. Lp(a) Catabolism

The question now arises as to how Lp(a) might be catabolized. Since it is not ethical to investigate lipoprotein uptake by different organs in humans in vivo, scholars performed such studies of the uptake of radiolabeled human Lp(a) into

different organs of laboratory animals, including mice, rats, rabbits, and hedgehogs, and found that the majority of Lp(a), approximately 50–60%, winds up in parenchymal liver cells [8]. The remainder was found in the bile, spleen, and kidney. This led us to speculate that many of the receptors mentioned above, to some extent, play a role in Lp(a) removal from circulation. More recently, two receptors that are not specific for lipoproteins, namely the asialoglycoprotein receptor (ASGP-R) [9] and the plasminogen receptor, both highly abundant on liver cells, turned out as strong candidates for their role in Lp(a) catabolism.

The ASGP-R is responsible for removing “aged” glycoproteins from circulation that might have been modified after a long residence time. In fact, many glycoproteins possess sialic acid as terminal sugar and, after its cleavage by neuraminidases, the penultimate sugar mannose-amines of galactose-amine get exposed and are strongly bound by the ASGP-R on liver cells and removed. This might also occur with Lp(a), as scholars were able to demonstrate that even native Lp(a) is bound to some extent to ASGP-R positive, but not by ASGP-R negative fibroblasts. These findings have also been verified by in vivo studies in rats. After treatment of Lp(a) with neuraminidase in vitro and injected into rats, scholars observed a very fast uptake and catabolism by the liver.

The second receptor of note is the Plg receptor, PlgRKT. The group of McCormick published an interesting work in 2017 in Circulation Research [10], providing strong evidence that the PlgRKT present on liver cells binds a great deal of Lp(a). This is not surprising since apo(a) is highly homologues to Plg. The most interesting results of these studies, however, were that Lp(a), after binding and internalization into lysosomes, dissociates into LDL, which is degraded. The liberated apo(a) migrates from Rab5+ early endosomes to the trans-golgi network and Rab11+ recycling endosomes and finally is secreted in an intact, un-degraded form. The recycled apo(a) probably re-assembles outside the liver with apoB-100, forming a new Lp(a). Since it is known that recycling proteins, such as transferrin and apoE, play physiological roles in transporting ligands into cells of specific organs, the authors speculated that this in-fact might be the function of apo(a), namely, to shuffle substances such as oxidized phospholipids or fibrin fragments into corresponding organs. The results of these experiments are highly relevant for interpreting the data of in vivo metabolic studies. It would mean that there exist two pools, one consisting of newly biosynthesized apo(a) and the other of recycled apo(a), and both apo(a) pools must have striking different metabolic parameters. It will be challenging to dissect these two pathways in future research and clarify their role in the overall metabolism under normal conditions and under the influence of different medications.

3. Lp(a) and Diabetes Mellitus (DM)

DM is a multifactorial and multigenetic disease and, as evidenced in the last decade, the characterization of patients with malfunctions of glucose (Glc) metabolism is far more complicated than originally thought. In the past, DM was mostly classified superficially and divided into two types: Type-1, characterized by the lack of insulin production, and Type-2, characterized by insulin resistance; alternatively, they were also frequently called juvenile diabetes mellitus and mature-onset diabetes mellitus. Today it knows that there are numerous facets found in both types of DM that are either genetically determined, acquired, or both. A key element in DM is the glucose concentration in blood under fasting conditions and post-prandially. Simply speaking, the blood-glucose concentration is a result of its rate of biosynthesis and its rate of catabolism. The metabolic pathways of Glc biosynthesis and secretion into the blood are textbook knowledge and may not be reiterated here. Concerning its catabolism, there are many pathways that must be considered, ranging from uptake into cells of various organs involving glucose transporters (GLUTs), some of them being insulin-dependent, the burning of Glc for energy supply, the excretion of Glc by the kidney into urine and many more. One can imagine that in all the anabolic and catabolic pathways, a wealth of enzymes and their corresponding genes are involved that impact the pattern of DM. A key element in regulating blood glucose, without a doubt, is insulin.

The pathomechanisms in T2DM are quite distinct from that of T1DM. The classical form of T2DM is characterized by hyperinsulinemia caused by the resistance of the relevant organs, muscles, and adipose tissue to take up Glc in response to sensing insulin. Based on contemporary genetic methods, close to 100 polymorphisms and mutations relevant for the etiology of T2DM have been identified, and this sheds some light on the complexity of this disease [11][12]. These features led C. Herder and M. Roden to recently propose a novel typology of DM [13]. They clustered the phenotypes of DM into five different diabetes subgroups, two relevant to T1DM and three to T2DM. Although this classification still represents an oversimplification concerning genotypes, they certainly will help to improve the differential diagnosis and treatment protocol for patients suffering from DM.

3.1. Lp(a) in T1DM

The data reported in Refs. [14][15][16] and state that: (1) There is no inherent effect on plasma Lp(a) levels caused by T1DM. (2) T1DM patients that are well-controlled have comparable Lp(a) levels to controls. (3) T1DM patients suffering

from microalbuminuria and, more strikingly, patients with kidney disease, have increased plasma Lp(a) levels and (4) physical activity and healthy lifestyle normalizes elevated plasma Lp(a) in T1DM patients who have normal kidney function.

3.2. Lp(a) in T2DM

The situation of Lp(a) in T2DM is far more complicated. Since both Lp(a) and T2DM are strong risk factors for atherosclerosis, one would expect that this might be reflected by elevated Lp(a) levels. However, publications consistently report lower plasma Lp(a) in T2DM patients compared to controls. Scholars call this the Lp(a) paradox in type-2 diabetes mellitus. T2DM is frequently accompanied by hypertension, altered lipid metabolism, elevated VLDL, hyperuricemia, hyperinsulinemia, inflammation, oxidative stress, as well as genetic polymorphisms in Glc transporters, nuclear receptors and more. All these factors have been shown to influence the metabolism of Lp(a).

3.3. The Lp(a) Paradox in T2DM

In the first investigations in 1981, regarding the role of Lp(a) in myocardial infarction, scholars found that high Lp(a) is not only a risk factor in normo-lipemic individuals but also to a much greater extent in individuals with elevated LDL-C [17]. In contrast, in individuals with Type-IV hyperlipoproteinemia, where patients consistently show an impaired Glc tolerance or T2DM, Lp(a) appeared to be a “negative risk factor,” i.e., MI patients had lower Lp(a) levels than controls.

In fact, it was found that carriers of the Q268X mutation not only suffer from MODY but also have reduced plasma concentrations of Lp(a), apoAII, and apoCIII. There are other mutations and polymorphisms known in the MODY genes that may have similar effects on plasma Lp(a). Of further relevance are the findings that T2DM patients show aberrations in hormones other than insulin, such as testosterone, IFG-1, or thyroid hormones, all of which are known to impact *APOA* expression [18].

In summary, it appears that T1DM patients have Lp(a) concentrations that are not different from healthy individuals if they are well-controlled and free of kidney dysfunction. T2DM patients, on the other hand, may have reduced Lp(a) due to mutations or polymorphisms in genes that affect the expression of the APOA gene on the one hand and the phenotype of DM on the other.

3.4. Lp(a) as a Risk Factor for CAD in Patients with DM

In theory, Lp(a) should be at least as atherogenic, if not more, in diabetic patients than in non-diabetics. Lp(a) contains large amounts of oxidized phospholipids, a hallmark of atherogenesis. Due to its longer residence time in the blood compared to LDL [19], Lp(a) is probably glycated to a larger extent than LDL, thus contributing to its atherogenicity. That this occurs in vivo is supported by the findings of Kotani et al. [20] who demonstrated impaired endothelial function likely related to oxidized Lp(a) from T2DM patients. The theoretical considerations mentioned above have also been corroborated in patient studies in vivo.

In 2006, Kollerits et al. [21] questioned to what extent Lp(a) might be an independent predictor of CVD in IDDM patients. More than 400 IDDM patients were followed over an observation period of 10.7 years. Since renal disease is a significant risk factor for CAD, patients with impairments of kidney function were excluded from the study. Although the study did not answer the question as to whether or not IDDM patients have increased Lp(a) levels, it was concluded that Lp(a) values >30 mg/dL contribute significantly to the CAD risk in T1DM. Similarly, calcified aortic valve disease was found to occur more frequently in T1DM patients with high Lp(a) [22].

There are numerous reports documenting that the situation in T2DM patients with respect to the atherogenicity of Lp(a) is very similar to that of T1DM. In one of them, Saeed et al. [23] examined the association of Lp(a) with the risk for CVD in close to 10,000 male and female participants, 1543 of them suffered from diabetes or pre-diabetes. From the results, the scholars concluded that “Adding lipoprotein(a) to traditional risk factors improved ASCVD risk prediction.” Interestingly, in a recent study by Markus et al. [24], it was reported that the relative increase in mortality from CVD was significantly higher in women with T2DM compared to men with T2DM.

Concluding from studies published so far, it appears that elevated plasma Lp(a) levels in T2DM patients positively correlates with the incidence of atherosclerotic cardiovascular disease. Despite the Lp(a) paradox in T2DM, there is no indication that lowering Lp(a) might negatively affect the cardiovascular outcome of this disease.

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