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Lipoprotein Lp(a) and Atherothrombotic Disease

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High plasma concentrations of lipoprotein (a) [Lp(a)] are now considered a major risk factor for atherosclerosis and cardiovascular disease. This effect of Lp(a) may be related to its composite structure, a plasminogen-like inactive serine-proteinase, apoprotein (a) [apo(a)], which is disulfide-linked to the apoprotein B100 of an atherogenic low-density lipoprotein (LDL) particle. Apo(a) contains, in addition to the protease region and a copy of kringle 5 of plasminogen, a variable number of copies of plasminogen-like kringle 4, giving rise to a series of isoforms. This structural homology endows Lp(a) with the capacity to bind to fibrin and to membrane proteins of endothelial cells and monocytes, and thereby inhibits binding of plasminogen and plasmin formation. This mechanism favors fibrin and cholesterol deposition at sites of vascular injury and impairs activation of transforming growth factor-beta (TGF-β) that may result in migration and proliferation of smooth muscle cells into the vascular intima. It is currently accepted that this effect of Lp(a) is linked to its concentration in plasma, and an inverse relationship between apo(a) isoform size and Lp(a) concentrations that is under genetic control has been documented. Recently, it has been shown that inhibition of plasminogen binding to fibrin by apo(a) from homozygous subjects is also inversely associated with isoform size. These findings suggest that the structural polymorphism of apo(a) is not only inversely related to the plasma concentration of Lp(a), but also to a functional heterogeneity of apo(a) isoforms. Based on these pathophysiological findings, it can be proposed that the predictive value of Lp(a) as a risk factor for vascular occlusive disease in heterozygous subjects would depend on the relative concentration of the isoform with the highest affinity for fibrin.

Key Words: Lipoprotein(a), Atherosclerosis, Thrombosis, Fibrinolysis.

Introduction

In 1963, Kâre Berg identified the presence of lipoprotein(a) [Lp(a)] in plasma (1); nevertheless, it was not until 1987 when Eaton et al. (2) partially identified the apoprotein(a) [apo(a)] glycoprotein sequence. The protein was later cloned (3) and showed a strong structural similarity between one of the Lp(a) components, apo(a) glycoprotein, and the plasmin precursor, plasminogen. This finding stimulated the interest of different research groups who found a common point between atherosclerosis and thrombosis (4–11). Different epidemiological studies were performed from this perspective and identified a positive correlation between high Lp(a) plasma concentration and an increase in cerebrovascular (12) and cardiovascular diseases, as well as coronary restenosis, postangioplasty reocclusion, and premature development of atherosclerosis related to high low-density lipoprotein (LDL) concentrations and/or low high-density lipoprotein (HDL) concentrations (13,14). Most prospective studies have confirmed these results (15–21). Even when a normal value for plasma concentration has not been agreed upon, some investigators considered this to be 20 mg/dL (20) and others suggested 30 mg/dL (15), pointing out that the use of different antibodies, monoclonal or polyclonal, generates differences in results (5).

Nevertheless, other studies do not find a relationship between Lp(a) and coronary arterial disease (22,23). This discrepancy may be a reflection of the large structural hetero-

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geneity of the apo(a) molecule, which is conducive to a functional heterogeneity as a plasminogen competitive inhibitor of fibrin because it lowers the formation of plasmin (7,24).

Not all organisms synthesize apo(a). Its presence has been identified in humans, in some Asian, European, and American primates (25), and in hedgehogs (26).

Function

The function of apo(a) in the organism is unknown. It provides cholesterol from the liver to organs that synthesize steroidal hormones (28) and to tissues for cell repair (29). Furthermore, the function of apo(a) found in testicles and brain is unknown and is independent from Lp(a); that is, it functions without being part of the Lp(a) molecule (30).

Concentration

Lp(a) plasma concentration, which depends on its hepatic synthesis (31,32), varies from one individual to another within an approximate range of <10 mg/dL to >100 mg/dL; it is independent on other factors such as diet, cholesterol, obesity, and smoking, and is maintained within small variations throughout the lifespan (33,34). In general, an individual inherits, in a codominant autosomic fashion, two apo(a) isoforms that may be identified by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and, subsequently, by immunotransference, making use of monoclonal or polyclonal antibodies against apo(a). By means of this method, almost 37 isoforms have been identified, which, according to the Utermann classification are: F (faster); B (i.e., apoB-100); S1 (slow), and S2, S3, or S4, depending on their migration velocity, in comparison with that of apoB-100 (35). The method does not allow for distinction of differences between isoforms of close molecular weight. Nevertheless, it has recently become possible to visualize the isoforms with the help of a reference, accounting for apo(a) recombinant isoforms with different molecular weights, correlating ($r = 0.97$) with the technique of pulsed-field electrophoresis (36). With the use of this reference it has been possible to obtain a linear relationship between the log r-apo(a) kringle number and the relative migration using SDS-PAGE, successfully identifying apo(a) isoforms over a wide range of molecular sizes. Pulsed-field electrophoresis has been employed to identify different genotypes codified for apo(a) (37). With the aforementioned technique, 19 different alleles have been described in a U.S. study of a population of whites (38).

Apo(a) isoform size accounts for an inverse correlation with Lp(a) plasma concentration (39), probably because, as apo(a) size increases, less protein is secreted from the cell, as occurs in the case of the human hepatocarcinoma cell line HepG2 (40).

Some hormones can modify Lp(a) plasma concentration, such as estrogens (41–44); anabolic steroids might reduce it (45) and growth hormone can increase it (46), but it cannot be modified by lipid-lowering medication or by diet (33,47). The plasma concentration of Lp(a) increases in diseases such as diabetes mellitus, nephrotic syndrome, rheumatoid arthritis, and in a transitory fashion after myocardial infarction or surgical intervention (5,33,34,48–50).

There are variations both in its average concentration and in the abundance of isoforms in genetically different populations (51–54). Lp(a) plasma concentration in blacks is at least three times greater than in whites (55–58).

Chemical Structure

Lp(a) composition is similar to that of LDLs. Both of these lipoproteins contain cholesterol, triglycerides, and phospholipids that may be dissolved and transported by plasma, due to the presence of a protein, apoB-100, which surrounds the lipid group and cholesterol (Figure 1).

The difference between them is that Lp(a) contains another glycoprotein, apo(a), which is bound to apoB-100 by a disulfide bridge between Cys in the 69 position for KIV-9 of apo(a) and Cys 3734 for apoB-100; the union is stabilized by hydrogen bonds and van der Waals interactions in other areas of both proteins (59–61). ApoB-100 (62) has the same structure and conformation in Lp(a) and the LDL molecule. The ratio apo(a):apoB-100 is 1:1 (63), and in considering their physicochemical variables there may be entities in a 2:1 ratio (64).

As usually occurs with some plasma proteins, Lp(a) may have different sizes with weights, ranging between 800 and 1300 kDa, and thus different densities. These differences are a reflection, although to a lesser extent, of lipid core composition, and especially of apo(a) structural polymorphism (65).

There are technical difficulties involved in obtaining apo(a) in its native form, and predictions of its secondary structure suggest an absence of an α-helix (66). Nevertheless, after reducing sulfhydryl binding, and by means of the circular dichroism technique, 8% α-helix, 21% β-sheet, and 71% random arrangements have been observed (67). Apo(a) belongs to the serine protease family, together with plasminogen, prothrombin, tissue plasminogen activator, urokinase-type plasminogen activator, and factor XII. These proteins derive from an ancestral gene common to all of them. Apo(a) is very similar to plasminogen. Genes codifying both proteins are very close, in chromosome 6, band q26-27 (35). The genes are a 50-kb distance apart in a head-to-head position (68,69) in the terminal region 5′. In the case of apo(a), the gene presents a polymorphism, which can be expressed by modifying the efficiency of the transcription. This, in turn, originates differences in Lp(a) plasma concentration, not only between individuals but between different ethnic groups (68). The apo(a) gene may have different sizes, each

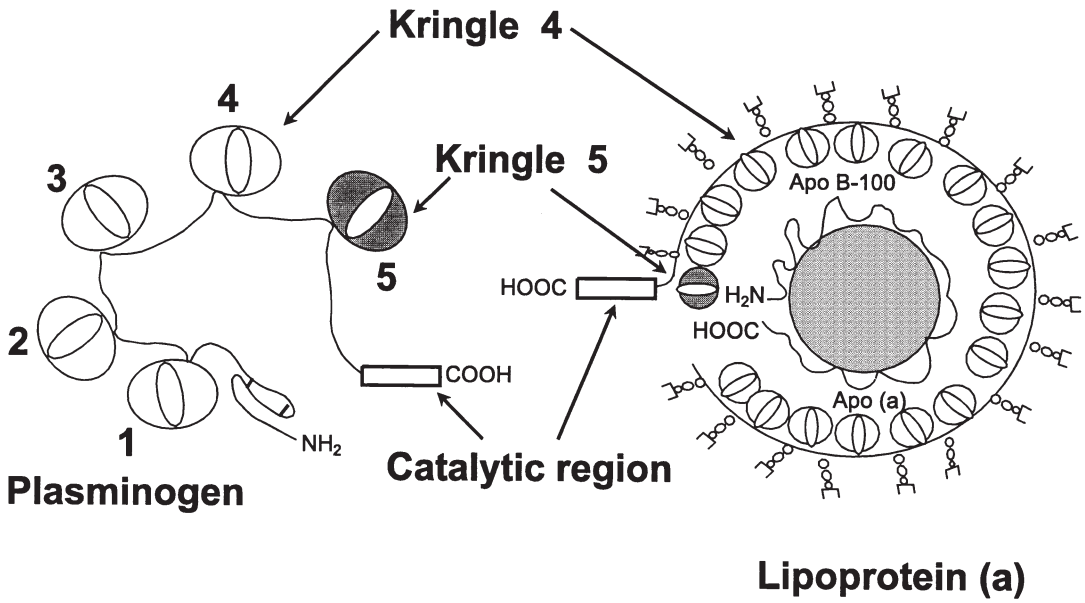

Figure 1. Chemical structure of lipoprotein(a) and plasminogen.

corresponding to the number of times in which a 5.5-kb sequence is present, with its number varying between 12 and 51 (3). The gene-promoting region of apo(a) possesses, in a distal position, a 1-kb fragment with a sequence showing sites with a different interaction potential. For interleukin-6 (IL-6), it shows seven sites; this conformation could explain the increase in Lp(a) plasma concentration during acute inflammation states and the three sites with specific elements (HNF-1, CEBP, and LF-A1) for hepatic transcription (69).

Apo(a) and plasminogen contain a protease region with a 94% similarity, but in the case of apo(a) it lacks the ability to become active and perform its enzymatic function due to the presence of arginine instead of serine at the activation site (3).

Plasminogen and apo(a) also include a different number of modules, called kringles. Through kringles they bind and recognize other macromolecules and/or specific sites in the cell membrane (73,74).

Kringles connect with each other by interkringle regions that are segments of 26–36 serine-, proline-, and/or threonine-rich amino acids (3); each interkringle region contains six potential O-glycosylation sites.

The kringle recognizance function includes the participation of a structure generated in the inner loop surrounding a hydrophobic region formed by different aromatic amino acids that are stabilized by means of hydrogen bonds and separate a cationic from an anionic group (73). This region is known as the lysine-binding site (LBS). Its structural characteristics generate a relatively rigid geometry that allows selective access and binding of 6.8-Å aliphatic or aromatic ligands of ω-amino-carboxylic acid type, such as ω-amino-hexanoic acid or similar compounds (74). Plasminogen comprises five very similar kringle types, but with small differences at the lysine-binding site, which modify the degree of affinity for different ligands. Plasminogen K I has a high-affinity LBS; the cationic pole has Arg-35 and Arg-71, and the anionic pole has Asp-55 and Asp-57 (74,75). The LBS in plasminogen K IV has an intermediate affinity; its V-shaped hydrophobic region generates a topography in which the aromatic rings of Phe-64, Trp-62, and Trp-72 separate the anionic group, which is formed by Asp-55 and Asp-57, and from the cationic group by Lys-35 and Arg-71 (76).

Apolipoprotein (a) Polymorphism

Apo(a) shares kringle V and a variable number of kringle IVs with plasminogen (35). Not all kringles IV of apo(a) are alike; they are classified into 10 different subtypes (77). In the apo(a) molecule, each is present only once, except for kringle IV-2, which appears on multiple copies, originating structural heterogeneity and different size isoforms that account for molecular weights of between 280 and 800 kDa.

Kringle IV-10 is most similar to the kringle IV of plasminogen, with the high-affinity, lysine-binding site being formed by Asp-55 and Asp-57 in the anionic pole, and by Arg-71 and Arg-35 in the cationic pole. Between the two poles there is a hydrophobic microenvironment that is formed by three aromatic amino acids: Trp-62, Phe-64, and Trp-72. KIV-10 of apo(a) differs from K-IV of plasminogen due to the presence of Arg instead of Lys in position 35. This kringle has a very important role in the Lp(a) union with lysine (78), preventing plasminogen access to the fibrin clot, thus blocking the action of the tissue plasminogen activator (79–82). This generates fibrinolytic insufficiency that, in turn, promotes atherosclerosis and thrombosis.

Another apo(a) polymorphism source is glycosylation;

each apo(a) KIV has a potential site for N-glycosylation. If interkringle glycosylation is considered, 30% of each mole of apo(a) corresponds to carbohydrates as follows: mannose; galactose; galactosamine; glucosamine, and sialic acid in ratios of close to 3:7:5:4:7, respectively (83).

Lp(a) Plasminogen Competitive Inhibitor

The fibrinolytic system destroys fibrin deposits in blood vessels, either those remaining from hemostatic activity or those formed and accumulated during the development of the atherosclerotic plaque. Because there is a balance between plasminogen activators and different types of inhibitors of these activators, its response varies either toward a static condition or a fibrinolytic activity.

As a result of fibrinolytic activation, plasmin is generated, a proteolytic enzyme that may have effects on different plasma substrates. In the present case, however, this does not occur, because fibrin itself locates and signals the fibrinolytic activation sites. Within the fibrin mesh, plasminogen is bound precisely at the lysine-binding site and permits and properly directs the binding of its tissue activator (i.e., tissue plasminogen activator). Therefore, plasmin is generated in the inner section of the fibrin deposit, breaking up and exposing other affinity sites such as terminal carboxylysine residues, to amplify its response. Once fibrin is disintegrated, the mechanism stops, and the vascular endothelium does not release additional activator; the remaining circulating plasmin and tissue activator find their corresponding inhibitors, α2-antiplasmin and type 1 tissue plasminogen activator inhibitor (PAI-1).

The similarity between plasminogen and apolipoprotein(a) allows different apo(a) isoforms to compete with plasminogen for fibrin affinity sites. The affinity of each isoform depends on its size and its plasma concentration (7,84,85); in addition, the plasmin formed at the surface of fibrin may vary with modifications of the concentration of Lp(a) *in vivo* (94).

Plasminogen and the different Lp(a) isoforms (86) also compete for lysine residues on the surface receptors of endothelial cells (81,87), U937 monocytes (87,88), platelets (89), mononuclear cells (86), and on matrices for *in vitro* models simulating the extracellular membrane (90).

Another mechanism that alters the fibrinolytic system balance is either the decrease or increase in synthesis of the tissue plasminogen activator (t-PA) or of the tissue plasminogen activator inhibitor (PAI-1), respectively (33,91), which is observed in cultures of endothelial cells exposed to Lp(a).

Lp(a) and Atherogenesis

Lp(a) favors atherogenesis through different mechanisms; macrophages phagocytize Lp(a) (92), migrate, and settle in the subendothelium, becoming transformed into foam cells, generating deformities that decrease the lumen of the blood vessels. It has been observed that, in cultures of endothelial cells from the coronary artery, Lp(a) stimulates the expression of vascular adhesion molecule-1 (VCAM-1) and selectin E, a process that triggers attraction to macrophages (93). Another proposed mechanism is related to a decrease in plasmin generation that accounts for: (a) prolonged permanence of fibrin deposits (74), with the consequent increase of cholesterol deposits and formation of atherosclerotic plaque, and (b) decrease of activation, by partial hydrolysis with plasmin, of TGF-β, which prevents the growth of vascular smooth muscle cells.

Lp(a) Inhibits Fibrinolysis Depending on Apo(a) Isoforms

A high plasma concentration of Lp(a) does not always interfere with normal fibrinolysis. Apo(a) isoforms show different antifibrinolytic activity (84,85), hence the importance of taking into account the antifibrinolytic activity of the isoforms in the prediction of cardiovascular diseases (95).

Many different strategies have been applied in *in vitro* studies. While making use of a solid-phase fibrin model (96–100), it has been possible to identify, with high specificity and sensitivity, fibrinolysis inhibition by apo(a) isoforms. These studies have permitted identification of the mechanisms and different variables involved in plasminogen competitive inhibition, due either to the different Lp(a) native isoforms (84) or through apo(a) recombinant forms (24).

These studies demonstrate the following: (1) Lp(a) affinity for fibrin shows an inverse relationship with the size of the apo(a) isoform within a K_d range of 50–500 nM (84); (2) both plasminogen and Lp(a) compete for the same binding sites, which corresponds to a saturable competitive inhibition mechanism, and (3) Lp(a) antifibrinolytic potential depends on the affinity and concentration of each of the two apo(a) isoforms found in plasma (101).

Lp(a) and Homocysteinemia

Hyperhomocysteinemia is related to an increase in the incidence of thrombotic and atherosclerotic diseases (102). Thrombosis is favored because of alterations in different antithrombosis-regulation mechanisms; in addition, it increases tissue factor activity, lowers the expression and activity of thrombomodulin necessary for protein C activation, and lowers the anticoagulant activity of antithrombin III as well as the binding capacity of the tissue plasminogen activator to its receptor on cell surfaces (anexin II) (103). In addition, an increased plasma homocysteine concentration manifests itself as an increase in Lp(a) binding to fibrin (104,105).

Conclusions

Most epidemiological studies point out the relationship between lipoprotein (a) plasma concentration and the risk of suffering cardiovascular and cerebrovascular diseases, espe-

cially when other risk factors are present, such as high levels of low-density lipoproteins and smoking. It is also important to point out that there are differences in the concentration and in the presence/nonpresence of some isoforms within genetically different populations. Therefore, identification of fibrinolysis inhibition behavior of different Lp(a) isoforms necessitates applying a strategy to the study that will allow for the identification of individuals and ethnic groups suffering from fibrinolytic deficiency due to the presence of high concentrations of Lp(a). This line of investigation represents a challenge to the various disciplines in studying the role of Lp(a) in the pathogenesis and progression of atherosclerosis.

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