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Review

Lipoprotein(a): New Insights into Mechanisms of Atherogenesis and Thrombosis

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Summary: Lipoprotein(a) (Lp[a]) continues to be a controversial molecule regarding its role in human vascular disease. Although the physiologic role of this molecule is still unclear, novel discoveries within the last few years have suggested numerous mechanisms whereby Lp(a) may contribute to atherosclerosis and its complications in human subjects. These effects may differentially occur in vascular tissue and circulating blood compartments. A complex interplay between tissue-specific effects is probably more relevant to the pathogenicity of this molecule than one single effect alone. This review briefly describes the structure of Lp(a) in relation to its biochemical function, summarizing the current literature on various pathophysiologic mechanisms of Lp(a)-induced vascular disease and the role of cell and tissue-specific effects in promoting atherogenesis and thrombosis.

Key words: lipoprotein(a), tissue factor pathway inhibitor, atherogenesis

Introduction

Since its discovery by Berg 40 years ago, lipoprotein(a) (Lp[a]) has been the subject of controversy and debate regarding its role in human atherosclerosis.[1] Although several retrospective studies have shown an association between Lp(a) excess and risk of coronary heart disease (CHD),[2–4] prospective studies including three clinical trials[5–7] have yielded conflicting results.[5–14] Results of positive studies seem to suggest that Lp(a) is an independent but weak predictor of CHD in unselected populations and a powerful predictor of premature CHD in patients with concomitant hypercholesterolemia.[15] Conflicting evidence in the literature has been attributed to several factors including small sample size, selection bias, lack of standardized Lp(a) assay methods, storage of plasma samples at temperatures too high to maintain Lp(a) integrity, and use of parametric rather than nonparametric methods to analyze this variable which is not normally distributed.[16] However, the preponderance of current evidence is in favor of Lp(a) as a risk factor for atherosclerosis and coronary heart disease.[15–18] Screening for elevated Lp(a) levels in a high-risk population is currently performed in many centers, and although effective therapies are currently unavailable, novel discoveries regarding the structure and function of this molecule suggest potential avenues for future drug design and targeting. This review will briefly discuss the structure and biology of Lp(a), followed by a step-wise discussion of how Lp(a) may induce atherogenesis and thrombosis, with particular emphasis on recent novel discoveries such as its effects on plaque stability and the tissue factor pathway.

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Lipoprotein(a): Structure and Biochemistry

Lipoprotein(a) can be broadly classified as a heterogeneous group of low-density lipoproteins containing two protein moieties, apolipoprotein (Apo) B-100 and Apo(a), linked by a disulphide linkage.[19] While Apo B-100 contains low-density lipoprotein (LDL), Apo(a) is a highly polymorphic glycoprotein ranging in size from 300 to 800 kD.[16] Apolipoprotein(a) consists of multiple kringle domains that have a close structural homology with plasminogen,[20, 21] with both genes being located on chromosome 6.[22, 23] The plasminogen cDNA contains a signal sequence, a tail region, five triple-folded disulfide linked loops called kringles, and a protease region. The Apo(a) molecule has 10 kringle domains similar to kringle IV of plasminogen (kIV types 1–10) and a single kringle similar to kringle V of plasminogen. Each of the 10 kringles resembling kringle IV of plasminogen is present in a single copy in the Apo(a) molecule except kringle 2, which is present in varying number of repeats both within and among individuals, giving rise to the size polymorphism of Apo(a).[24, 25]

The kringles on Apo(a) serve important functions (e.g., kringle IV type 10 is responsible for the strong lysine binding properties of Lp[a]).[26, 27] Other kringles play important pathobiological roles, such as interacting with scavenger receptors on foam cells and in the formation of Lp(a).[16] Enzymes of the elastase family cleave Apo(a) in the region between kringle IV and kringle V to yield F1 and F2 fragments representing the amino and C terminal ends of the molecule, respectively.[28, 29]

In a similar fashion, elastase cleaves Lp(a) in the same region to form F1 fragment and mini Lp(a), which is the F2 fragment connected to the LDL particle. In vivo studies suggest that the F2 fragment is retained within the atherosclerotic plaque and is a potential cause for the atherothrombogenic action of Lp(a),[1] whereas the F1 fragments may return to the circulation. Furthermore, following intravenous administration of Lp(a), F1-derived Apo(a) fragments can be isolated from plasma and urine of humans and mice.[30] Indeed, enzymes such as metalloproteinases and elastases present within the atherosclerotic plaque may contribute to this process and hence pathogenicity of Lp(a) by breaking it down into its F1 and F2 fragments.[31]

Mechanisms of Lipoprotein(a)-Induced Atherogenesis

Induction of Adhesion Molecules on Vascular Endothelial Cells

Lipoprotein(a) is believed to promote atherosclerosis by a number of separate but related mechanisms which are summarized in Fig. 1. Expression of adhesion molecules, VCAM-1 and E-selectin, on cultured human coronary endothelial cells is increased in the presence of Lp(a).[32] It also induces human vascular endothelial cells to produce monocyte chemotactic protein (MCP), a potent chemoattractant for monocytes and a key cytokine implicated in the pathogenesis of atherosclerosis.[33]

Atherosclerosis is increasingly believed to be an inflammatory disease,[34] and recruitment of monocyte macrophages is an important early step of atheroma formation.[35] Within days or weeks of feeding mice a high-fat and high-cholesterol diet, monocytes can be observed adhering to the surface of endothelial cells.[36] The monocytes then migrate into the arterial intima where they differentiate into macrophages and take up lipoprotein to form foam cells, an early pathologic marker of atherosclerotic plaque. Cell surface adhesion molecules such as intercellular cell adhesion molecule (ICAM)-1, E-selectin, and vascular cell adhesion molecule (VCAM)-1, play a key role by adhering to corresponding receptors on leukocytes such as LFA-1. Moreover, mice deficient in ICAM-1, P-selectin, CD18, or combinations of these molecules have been shown to develop smaller atherosclerotic lesions after lipid feeding.[37] In addition, higher levels of soluble VCAM-1 and E-selectin have been found in subjects with hypercholesterolemia.[38]

Enhancement of expression of cell surface adhesion molecules could be an important mechanism of Lp(a)'s atherogenecity. Other investigators have shown that Lp(a) enhances expression of ICAM-1 in cultured human umbilical vein endothelial cells (HUVEC),[39] although a similar effect with VCAM or E-selectin has not been found. Moreover, neutralizing transforming growth factor beta (TGFβ) antibodies enhanced ICAM expression in HUVECs, while addition of recombinant TGFβ inhibited the enhancement of ICAM-1 expression in Lp(a)-treated HUVECs, suggesting that enhancement of ICAM-1 expression by Lp(a) could in part be due to inhibition of TGFβ. This TGFβ-mediated effect of Lp(a) has been well documented both in vitro and in vivo in animal models and in human subjects by Grainger *et al*. and provides a theoretical basis for Lp(a) effects on smooth muscle proliferation within the vessel wall.[40–42]

Formation of Foam Cells and Atherosclerotic Plaque

Atherosclerotic plaques but not normal human arteries contain Lp(a). Plasminogen-like lysine binding sites present on

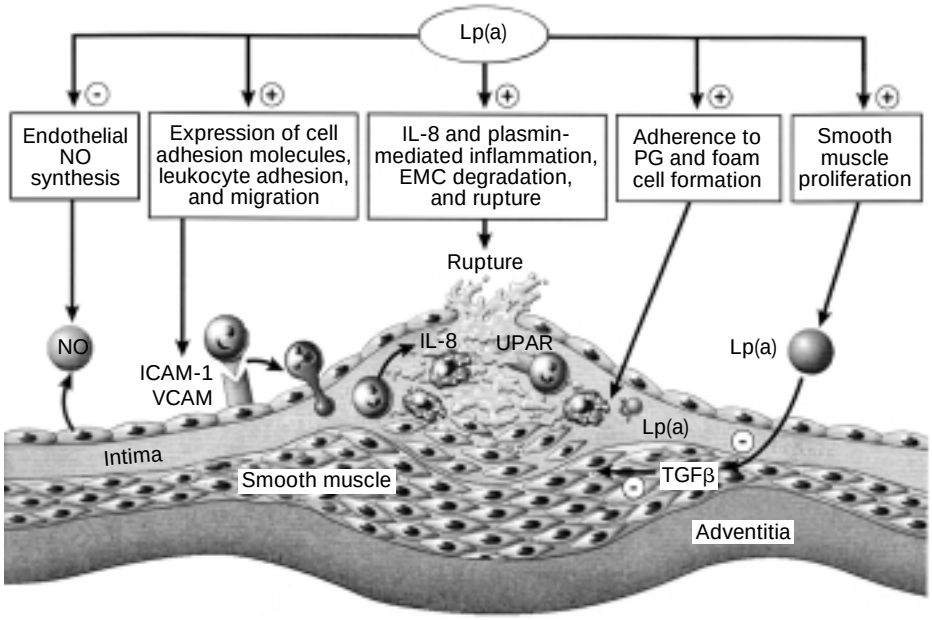

FIG. 1 Lipoprotein(a) (Lp[a])-mediated mechanisms of atherogenesis. Lipoprotein(a) has numerous effects on the nitric oxide (NO) pathway, cell adhesion molecules, and matrix degradation in addition to growth regulatory molecules. ICAM = intercellular cell adhesion molecule, VCAM = vascular cell adhesion molecule, UPAR = urokinase plasminogen activator receptor, ECM = extracellular matrix, PG = plasmin generation, TGFβ = transforming growth factor beta.

the Apo(a) molecule may play a key role in anchoring Lp(a) to the extracellular matrix within the arterial wall.[43] Mutations affecting the lysine binding sites of kringle IV-10 of Apo(a) have been shown to decrease affinity of Lp(a) to the vessel wall.[44] Transgenic mice expressing mini Apo(a) containing a mutation in kringle IV-10 lysine binding sites have significant reduction in fatty streak formation and Lp(a) accumulation within the vessel wall.[45] Klezovitch *et al.* have also demonstrated that proteoglycans within the vessel wall may play an important role in Lp(a) retention within the vascular intima.[46] In these experiments, Apo(a), via its C-terminal domain, was found to bind to the protein core of the proteoglycan decorin, a proteoglycan synthesized by vascular endothelial and smooth muscle cells[47–49] and present within atherosclerotic plaques.[50] This binding was shown to be hydrophobic in nature and not dependent on the lysine binding sites on the Apo(a) molecule. However, the nature of the interaction between decorin and intact Lp(a) was an electrostatic binding of the glycosaminoglycan (GAG) portion of decorin to the ApoB100 of Lp(a). This decorin-Apo(a) interaction has recently been proposed by Klezovitch *et al.* as an explanation for preferential vessel wall retention of Lp(a) over LDL.[46]

Within the diseased arterial wall, Lp(a) probably undergoes oxidative, proteolytic, and lipolytic changes induced by enzymes present within an atherosclerotic plaque, such as metalloproteinase, elastase, sphingomyelinase, and phospholipase. Oxidative modification by malondialdehyde, for instance, produces avid Lp(a) uptake by human monocyte-macrophages.[51] Cholesterol loading of macrophages also results in marked enhancement of Lp(a) and Apo(a) internalization and degradation,[52] revealing a lipid-driven mechanism for Lp(a) foam cell formation. Incubation of bovine aortic smooth muscle cells with Lp(a) in the presence of lipoprotein lipase and sphingomyelinase lead to massive aggregation of Lp(a) on the surface of these cells,[53] whereas coincubation with chondroitin ABC lyase prevented this aggregation, suggesting a key interaction with cellular proteoglycans. Moreover, coincubation of Lp(a)-coated smooth muscle cells with mouse peritoneral macrophages led to formation of lipid-laden macrophages on the surface of these cells with disappearance of visible Lp(a) aggregates. This could be an important interaction between Lp(a), smooth muscle cells, and macrophages, leading to foam cell and plaque expansion.

Plaque Inflammation and Instability

Novel mechanisms of Lp(a)-mediated plaque instability have been described recently. Human THP-1 macrophages produce interleukin-8 (IL-8) in the presence of Lp(a), an effect primarily mediated by the C-terminal region of Apo(a).[54] Interleukin-8 is a key inflammatory cytokine within atherosclerotic plaques[55] and possesses chemotactic activity toward neutrophils,[56] T cells,[57] monocytes,[58] and smooth muscle cells[59, 60] while decreasing macrophage expression of tissue inhibitors of metalloproteinases.[61] Disinhibition of metalloproteinases that cleave Apo(a) into F1 and F2 fragments[31] may increase inflammatory activity within plaque leading to rupture.

The expression of urokinase and urokinase receptors on monocytes is also increased in a dose-dependent manner,[62] resulting in increased plasmin generation. Increased protease availability may have multiple effects, including facilitation of cell migration and growth within plaque.[63] Furthermore, monocyte adhesion to extracellular matrix (ECM) is facilitated by increased expression of micro PAR and ICAM-1, receptors for vitronectin and fibrinogen, respectively. Increased monocyte adherence to ECM and enhanced plasmin and urokinase activity could be important mechanisms of Lp(a)-mediated ECM degradation and plaque rupture.

Vascular Cell Proliferation

The induction of human smooth muscle cell proliferation by Lp(a) was first demonstrated in vitro by Grainger *et al.*[41] This group showed that Lp(a) decreased generation of active TGFβ, an endogenous inhibitor of smooth muscle cell migration. Bovine pericytes and smooth muscle cells secreting TGFβ have also been shown to inhibit endothelial cell migration and repair of a denuded portion of a vessel in vitro.[64] Antibodies to TGFβ1 abrogated the above-mentioned inhibition, as did inhibitors of plasmin formation. This TGFβ effect is mediated by inhibition of plasminogen activation at the cell surface with subsequent inhibition of plasmin-mediated TGFβ activation.[64, 65] Furthermore, inhibition of TGFβ activation has been observed in Apo(a) transgenic mice[40] and in human subjects with elevated Lp(a). Apo(a) transgenic mice have been shown to have threefold less active plasmin and significantly less active TGFβ within the aortic wall than normal mice. Although the total TGFβ concentration was similar in sera from Apo(a) transgenic and normal mice, the proportion of total TGFβ in active form was significantly lower in the serum of Apo(a) transgenic mice. This TGFβ mechanism of dysregulated growth induced by Lp(a) is an attractive hypothesis for its effects on plaque growth.

Inhibition of Nitric Oxide and Endothelial Dysfunction

Nitric oxide (NO) has several pleiotropic antiatherogenic properties, including inhibition of T cell and smooth muscle proliferation,[66] neutrophil adhesion,[67, 68] platelet activation,[69] and reduction in endothelial permeability.[70] It is not surprising that decreased NO synthesis has been associated with atherosclerotic lesion development.[71, 72] Oxidized Lp(a) induces dose-dependent reduction of inducible nitric oxide synthase (iNOS) protein expression and mRNA synthesis in lipopolysaccharide/interferon-stimulated mouse macrophages.[73] Dose-dependent inhibition of iNOS by Lp(a) may lead to increased atherogenesis. Elevated Lp(a) levels have also been associated with impaired endothelium-dependent vasodilatation in coronary arteries.[74] In hypercholesterolemic children, flow-mediated dilation of the superficial femoral artery was inversely related to Lp(a),[75] and in patients with elevated Lp(a) levels an increased vasoconstrictor response occurs after administration of L-NMMA, an NO synthase inhibitor.[76] These combined effects suggest a compensatory increase in basal NO production by the endothelium in response to elevated Lp(a) levels.

Mechanisms of Thrombosis

Lipoprotein(a) may promote a more thrombotic state by a number of mechanisms, including inhibition of the fibrinolytic system and enhancement of the tissue factor-mediated pathway. These effects are illustrated in Fig. 2.

Inhibition of Plasmin Generation

Apolipoprotein(a), as discussed previously, has significant structural homology with plasminogen. A varied number of cell types have been found to express cell surface receptors for plasminogen. Both intact Lp(a) and recombinant Apo(a) inhibit plasminogen binding to endothelial cells,[77] U937 cells,[78, 79] and platelets.[80] The assembly and activation of plasminogen on the endothelial cell surface has been studied extensively, and it is known that plasminogen binds to the surface of endothelial cells via a tissue plasminogen activator (t-PA)/plasminogen coreceptor, identified as a member of the annexin superfamily of proteins.[81] In particular annexin II, which is selectively expressed on the endothelial cell surface,[81, 82] possesses independent binding domains for plasminogen and t-PA.[83, 84] Plasminogen appears to bind to the annexin receptor in a two step process, whereby the N-terminal glutamine-plasminogen is converted to N-terminal lysine-plasminogen by cleavage of a 76 amino acid preactivation peptide[85, 86] with subsequent activation of the receptor in the second step.[81] Tissue plasminogen activator binds to annexin at a separate site in close proximity to the plasminogen-binding site, leading to more efficient generation of plasmin.[87] Lipoprotein(a) inhibits generation of plasmin on the endothelial cell surface without interfering with t-PA binding,[88] and in a similar manner, Apo(a) inhibits plasminogen binding to annexin but has no effect on t-PA binding.[85] Decreased plasminogen binding on the cell surface may therefore create an antifibrinolytic state.

In addition, plasminogen activation by both streptokinase[89, 90] and t-PA[91] has been shown to be impaired in the presence of Lp(a), and mice transgenic for Lp(a) are resistant to t-PA-mediated lysis of artificially induced fibrin thrombi.[92] The mechanism for this action is believed to be in competition with plasminogen for binding to fibrin. It is interesting that plasmin catalyzes the binding of Lp(a) to immobilized fibrinogen and fibrin.[93]

The antifibrinolytic effect of Lp(a) is primarily defined by the size of the Apo(a) polymorphs, which display heterogeneity in their fibrin-binding activity.[94] The affinity of each isoform depends on its size and plasma concentrations, with smaller size isoforms displaying higher affinity binding to fibrin.[95, 96] The population most at risk for thrombosis, therefore, appears to be that possessing a predominant low molecular weight phenotype with high affinity for fibrin. Moreover, the Lp(a) phenotype (i.e., affinity for fibrin) may be more important as a determinant of risk than the actual plasma concentration of Lp(a).[97]

Increased Expression of Plasminogen Activator Inhibitor

Endothelial cell synthesis of plasminogen activator inhibitor-1 (PAI-1) is also increased by Lp(a).[98] In cultured human endothelial cells, Lp(a) enhanced PAI-1 antigen activity and mRNA expression without altering t-PA activity. In addi-

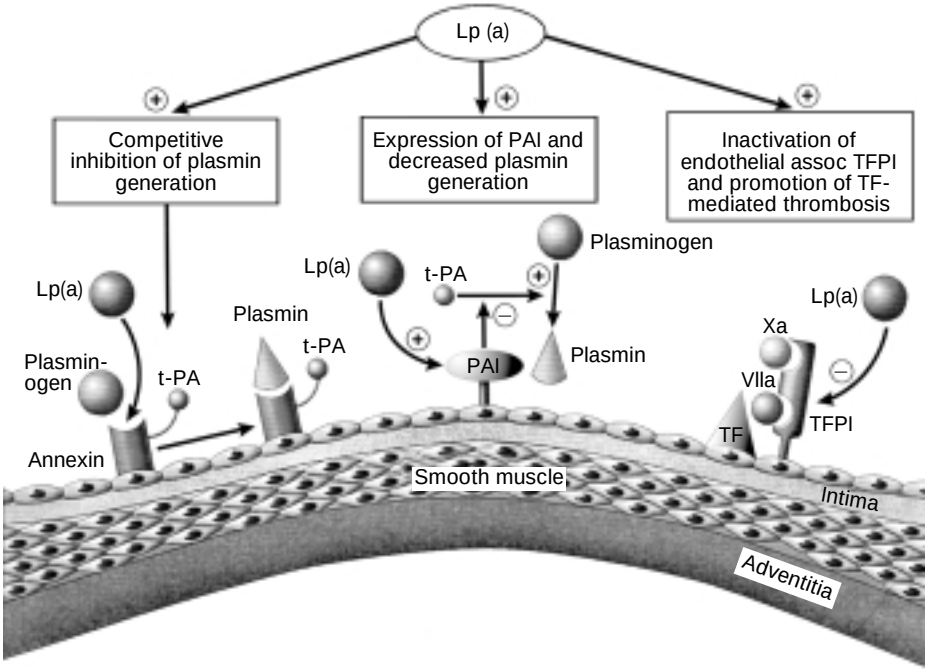

FIG. 2 Lipoprotein(a) (Lp[a])-mediated mechanisms of thrombosis. Lipoprotein(a) interacts with the fibrinolytic and coagulation systems potentiating tissue factor-mediated thrombosis and inhibiting clot lysis. PAI = plasminogen activator inhibitor, t-PA = tissue plasminogen activator, TFPI = tissue factor pathway inhibitor.

tion, monocytes derived from male patients with isolated Lp(a) hyperlipidemia, compared with those from healthy donors with normal Lp(a) levels, had increased upregulation of PAI-2 mRNA and protein.[99] This effect was gender specific, with no difference noted among females. Monocyte expression of PAI-2 was also increased, another potential mechanism of an antifibrinolytic effect. Together these data suggest a mechanism whereby Lp(a) inhibits fibrinolysis at the endothelial cell surface and promotes thrombosis.

Inhibition of Tissue Factor Pathway Inhibitor

Tissue factor pathway inhibitor (TFPI) is a Kunitz type serine protease inhibitor and a potent inhibitor of the tissue factor-mediated coagulation cascade.[100] Tissue factor pathway inhibitor is present on endothelial cells, activated monocytes, and platelets.[101–104] However, the endothelium is believed to be the principal site of synthesis of TFPI.[101] Tissue factor pathway inhibitor is expressed by vascular smooth muscle cells within atherosclerotic plaques,[105] and TFPI within atherosclerotic plaque is associated with reduced tissue factor activity within the plaque.[106] We have recently shown that Lp(a) binds and inactivates TFPI,[107] potentially augmenting unopposed tissue factor (TF) effects. Lipoprotein(a) can bind and inactivate recombinant as well as cell-associated TFPI in vitro in a dose-dependent manner. The LDL portion of Lp(a) isolated by dithiothreitol (DTT) reduction and gradient ultracentrifugation did not bind rTFPI, suggesting that this portion of Lp(a) was not important for binding. Apolipoprotein(a) bound to rTFPI in a similar concentration-dependent manner as Lp(a). It is interesting that lysine plasminogen (L-Plg) was also found to bind to immobilized rTFPI but was inhibited by nanomolar concentrations of Apo(a) demonstrating a binding affinity that was lower than that of Apo(a). Furthermore, this dose-dependent inactivation of TFPI by Lp(a) in vitro and on endothelial cell surfaces was not affected by plasminogen. No Lp(a) dose-dependent binding was seen when mutated forms of TFPI lacking the K3 domain or C terminus were immobilized instead of full length rTFPI demonstrating the importance of the C-terminal region of TFPI for this interaction. The binding of Apo(a) to rTFPI was shown to be lysine dependent and was inhibited by epsilon aminocaproic acid (EACA). This significant binding and inactivation of cell associated and recombinant TFPI by Lp(a) adds a further prothrombotic layer to the pleiotropic effects of this molecule. Thus, inhibition of TFPI within plaque at the endothelial surface and in the circulation may have additive effects in promoting thrombosis at the site of plaque rupture.

Vascular Tissue and Circulatory Effects

Discovery of new mechanisms suggest that the effects of Lp(a) within the vessel wall might be more relevant to its pathogenicity and different from its effects on circulating blood. Such differential effects might be a potential explanation for blood Lp(a) levels often not correlating with the incidence of coronary events. Cleavage of Lp(a) into potential atherogenic fragments, its retention by proteoglycans, and its induction of macrophage IL-8 expression are specific vessel wall atherogenic effects not seen to occur in peripheral blood. Moreover, Lp(a) causes increased plasmin activity within a plaque while decreasing circulating plasmin activity. These differential effects on blood and vessel wall may be additive in terms of atherothrombotic risk, potentially facilitating plaque rupture and later thrombosis on the luminal surface of the vessel.

Conclusion

A complex interplay of mechanisms affecting both vasculature and circulation contributes to the pathogenicity of this molecule. Novel discoveries elucidating such pathophysiologic mechanisms have provided us with multiple potential targets for drug design and therapy.

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