

E3. VASCULAR BIOLOGY

Lp(a) and the Microvasculature

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Lp(a) is a low density lipoprotein (LDL)-like particle that contains the apo B-100 portion of LDL in disulfide linkage to apolipoprotein(a) [apo(a)]. Apo(a) shares an extraordinary degree of homology with plasminogen including multiple tandem repeats of domains similar to kringle 4, a single region similar to kringle 5 and an inactive protease segment in which the potential activation site contains an arginine to serine substitution.^(1,2) The apo(a) and plasminogen genes are closely linked on chromosome 6, which contains at least two other plasminogen-related genes or pseudogenes.⁽³⁾ At present at least six members of the plasminogen gene family have been identified.^(4,5)

A number of epidemiologic studies have demonstrated a close correlation between increased blood levels of Lp(a) (above 30 mg/dL) and coronary and cerebral vascular disease.^(6,7) Coronary artery disease increases with elevated levels of Lp(a), an interaction that is independent of the plasma LDL or cholesterol level. Lp(a) is an independent risk factor for coronary atherosclerosis as well as saphenous vein bypass occlusion and correlates significantly with the severity and extension of angiographically detectable coronary lesions.⁽⁸⁾ In cardiac transplant patients, elevated Lp(a) appears to be an independent risk factor for accelerated coronary atherosclerosis.⁽⁹⁾ In this situation, Lp(a) in the vessel wall may facilitate or exaggerate cytokine-mediated inflammatory responses and alter the nonthrombogenic phenotype of the vessel. Ample quantities of Lp(a) have been identified by immunohistochemical techniques in atheromatous lesions.⁽¹⁰⁾ This includes dramatic depositions on the thickened intimal endothelial cell surface. The striking homology of apo(a) with plasminogen, a critical component of the endothelial cell membrane plasmin generating system, suggests that Lp(a) modulation of vessel wall function leading to atherogenesis occurs in the setting of altered fibrinolysis at the intimal surface due to competition of Lp(a) with plasminogen.

Endothelial Cell Plasmin Generating System

A significant body of experimental evidence, in fact, suggests that plasmin generating systems assemble on different cell surfaces and influence cell behavior in various pathophysiologic settings including inflammatory processes, tumor metastasis, ovulation, implantation and embryogenesis. On endothelial cells, plasminogen and t-PA assemble together on membrane-associated annexin II and enhance the generation of active protease at the cell surface.^(11,12)

Circulating N-terminal glutamic acid plasminogen (Glu-PLG) is converted to membrane-bound n-terminal lysine plasminogen (Lys-PLG) by cell surface plasmin proteolysis of a 76 amino acid preactivation peptide.⁽¹³⁾ Lys-PLG has an increased binding affinity for endothelial cells compared to Glu-PLg and is more efficiently converted to plasmin by t-PA on the endothelial surface. Lys-PLg appears to be the preferred zymogen ligand for the annexin II-binding site. Plasminogen binding to

membrane annexin II requires kringle-related lysine-binding sites that interact with receptor-associated carboxyl terminal lysine residues.⁽¹¹⁾ Lys-PLG has been identified using immunohistochemical techniques on the endothelial surface of blood vessels in various inflammatory tissues. Lys-PLG has also been detected on macrophages in chronic inflammatory sites and on intraductal breast carcinoma cells.⁽¹⁵⁾ These latter observations suggest that surface plasmin on some tumor cells may reflect a more malignant phenotype.

T-PA binding to membrane annexin II is specific, saturable, dependent on a linear sequence in the fibronectin like “finger” domain, and independent of lysine sites.⁽¹²⁾ It is of some interest that t-PA binding appears to be dependent on thiol-sensitive disulfide linkages as evidenced by the fact that cell-associated t-PA activity is markedly decreased by elevated homocysteine levels.⁽¹⁶⁾ This may explain in part the thrombogenic phenotype in some patients with homocysteinemia.

Annexin II, a multidomain amphipathic phospholipid-binding protein, represents a unique membrane site for the fibrinolytic assembly system. Membrane translocation of the intracellular protein occurs through microvesicular extrusion.⁽¹⁷⁾ Plasminogen binding appears to require plasmin cleavage at Lys-Arg (307-308) of the intact membrane associated protein.⁽¹¹⁾ Thus the annexin II assembly system represents a unique protease-modulated assembly in which plasmin or generation of a plasmin-like protease activates the binding site by generating a carboxyterminal lysine and also activates the ligand by generating a lysine end terminal on plasminogen converting the zymogen to the activated state. A number of different plasminogen-binding sites have been previously described including fibrin and GPIIb IIIa integrin on platelets,⁽¹⁸⁾ a-enolase on monocytoid cells,⁽¹⁹⁾ Heymann nephritis antigen on renal epithelium⁽²⁰⁾ and a streptococcal wall protein.⁽²¹⁾ Similarly, a number of different t-PA binding sites or receptors have been described on macrophages and hepatoma cells. Annexin II is the first clearly defined endothelial surface protein that specifically binds both t-PA and plasminogen and thus facilitates local generation of plasmin at the surface of the blood vessel wall.

The Lp(a) Effect

Lp(a) directly influences the endothelial cell plasmin generating function by competing for the annexin II plasminogen binding domain. Using cultured human endothelial cells, we have demonstrated that Lp(a) effectively competed with plasminogen binding and down-regulated cell surface plasmin generation.⁽¹⁰⁾ In an ELISA solid phase binding assay using purified annexin II, Lp(a) had no effect on t-PA binding but significantly inhibited plasminogen binding confirming the multidomain nature of the membrane-binding protein.⁽²²⁾ The approximately 90% inhibition of plasmin generation reflects a significant shift to a more thrombogenic phenotype at the vessel surface. In addition, Lp(a) may act as a competitive inhibitor of t-PA in the presence of fibrinogen and also interferes with the binding of plasminogen to fibrin clots.⁽²³⁾ Human apo(a) transgenic mice are relatively resistant to in vivo t-PA-mediated thrombolysis.⁽²⁴⁾ The atherogenic potential of Lp(a) may be enhanced by binding to the LDL receptor. Transgenic mice with overexpressed LDL receptors catalyze Lp(a) rapidly.⁽²⁵⁾ Although the cellular mechanisms are not fully clarified, it appears that Lp(a) targets LDL

deposition locally in tissues and in this manner further contributes to atherogenesis. It is of note that oxidized Lp(a) results in an enhanced interaction with macrophage scavengers and plasminogen-binding sites.⁽²⁶⁾

In addition to the effect on vascular wall constitutive plasmin generation and vascular lipid deposition, Lp(a) appears to modulate vascular function. Using immunohistochemical techniques, microvascular Lp(a) deposition has been demonstrated in a number of acute inflammatory states including Crohn's disease, gall bladder fistula, granulomatous lymph node and pericarditis.⁽¹⁵⁾ It is of interest that Lp(a) has been detected in the spiral arteries undergoing fibrinoid degeneration in the placenta of preeclamptic women.⁽²⁷⁾ Conspicuous Lp(a) deposition has been noted in the vasa vasorum of diseased coronary arteries. Lp(a) is not detectable in the microvasculature of normal tissues. It would seem that Lp(a) may modulate inflammatory cytokine vascular wall interactions. A number of recent observations have further substantiated the relationship of Lp(a) to vascular pathophysiology. Long-term competition of plasminogen binding to the annexin II assembly site on the vascular wall unbalances the constitutive nonthrombogenic phenotype of the healthy endothelium by down-regulating plasmin generation. The resulting impaired activation of vascular TGF contributes to localized smooth muscle proliferation.⁽²⁸⁾ Lp(a) deposition further alters the state of vascular activation by generating the adhesive glycoprotein ICAM-1⁽²⁹⁾ and the protease inhibitor PAI-1⁽³⁰⁾ while decreasing EDRF production.⁽³¹⁾ This constellation of cellular and molecular changes contributes to the generation of a chronic thrombogenic phenotype, promoting intimal fibrin deposition. Fibrin in turn acts on the vessel wall, stimulating mitogenesis and synthesis of collagen and proteoglycans. As fibrin is proteolyzed, chemotactic peptides are generated that contribute to inflammatory intimal cellular influx.+ It is of interest that Lp(a) itself co-localizes with the fibrin in atherosclerotic lesions and thus targets deposition of apo(B) lipoprotein, directly contributing to plaque formation.⁽³³⁾

The Plasminogen Gene Family and Angiogenesis

Plaque fissuring leading to plaque disruption and thrombosis triggers most coronary events. Neovascularization of atherosclerotic plaques in coronary arteries has been considered a major factor in plaque rupture associated with vascular spasm and intramural hemorrhage.⁽³⁴⁾ For this reason there is a renewed interest in the relationship of angiogenesis and atherosclerosis. Recent quantitative histomorphological studies have documented significant intraplaque angiogenesis comparable to that seen in tumors or wounds.⁽³⁵⁾ The prominent deposition of Lp(a) in inflammatory microvasculature as well as in the vasa vasorum of diseased coronary arteries raises the interesting possibility that Lp(a), a plasminogen like molecule, may initiate angiogenic events. Recent data strongly suggest that several members of the plasminogen gene family are important modulators of the angiogenic response. Angiostatin, a kringle 1-4 proteolytic fragment of plasminogen of 38kd blocks neovascularization and growth of metastases by directly increasing apoptosis in tumor cells.^(36,37) Hepatocyte growth factor containing four separate plasminogen-like kringle domains stimulates endothelial cell proliferation and motility, is a potent angiogenic factor, and has been categorized as a "multifunctional cytokine."⁽³⁸⁾

Apo(a)-related gene C (apo(a) rg-C), an apo(a)-related gene adjacent to the plasminogen gene on chromosome 6, codes for a polypeptide with an estimated five kringle domains homologous to plasminogen kringle 4.⁽³⁹⁾ The function of the protein synthesized in the liver is unknown, however. It is of interest that the transcript has been detected in breast tumor samples. Plasminogen-related gene-B (PRG-B) on chromosome 2 codes for an 8800 dalton polypeptide that is almost identical to the plasminogen preactivation peptide released with the conversion of glu-plasminogen to lys-plasminogen.⁽⁴⁰⁾ The function of the polypeptide is not known, but it is also expressed in cancer cells. The role of the plasminogen gene family (Table 1) in vascular physiology remains to be determined. We speculate that Lp(a), by altering vessel wall reactivity and initiating vasa vasorum proliferative responses, could markedly influence the stability of a coronary atherosclerotic plaque.

Clinical Considerations

Lp(a) plasma concentrations exhibit extremely wide variations between individuals. The levels are relatively unaffected by environmental factors such as diet and are determined almost exclusively by genetic factors. Catabolic rates among individuals do not vary significantly. Fifty to seventy percent of the individual variability in Lp(a) levels is linked with isoform size variation.⁽⁴¹⁾ A polymorphism in the 5 flanking region of the apo(a) gene appears to significantly influence translational efficiency.⁽⁴²⁾

A number of different potential therapeutic agents have been reported to lower circulating Lp(a) levels (Table 2). Niacin in relatively large doses, up to several grams daily, may be effective in some patients. Elevated LDL levels appear to elicit or exacerbate the risk engendered by circulating elevated levels of plasma Lp(a). Diet and exercise as well as standard pharmacologic approaches to lower plasma LDL levels may be the most efficacious approach to treat Lp(a)-initiated atherogenesis. These include HMGCoA reductase inhibitors, fibrin acid derivatives and ion exchange users. Experimental approaches have included chronic extracorporeal apheresis.

Table 1. Plasminogen gene family.

Plasminogen	Gene	Reference source	Chromosome	Kringle Region
Apolipoprotein (a) Hepatocyte	6	5	4	not found
Growth Factor	7		1-4	
Macrophage Stimulating Protein		3		1-4
PRG-B	2			none
Apo(a) rg-C	6		4	

Table 2. Lp(a) lowering agents.

1. Estrogen
2. Tamoxifen
3. Niacin

4. Gemfibrozil
5. Omega-3 fatty acid
6. N-acetyl cysteine
7. Prednisone
8. Neomycin

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