LIPOPROTEIN(A): ON THE CUTTING EDGE OF OCCAM’S RAZOR

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Based on epidemiological studies, lipoprotein(a) (Lp(a)) has been recognized as an emerging risk factor for cardiovascular disease. However, challenging questions remain with respect to a mechanistic definition of the role of Lp(a) in atherothrombotic disease, issues in measurement of Lp(a) levels in the clinic, and the role of Lp(a) in clinical practice.

Introduction and Brief Background

Lp(a) was identified in 1963 [1] and constitutes a unique lipoprotein class that contains an LDL-like moiety to which the unique glycoprotein apolipoprotein(a) (apo(a)) is covalently bound. The hallmark of apo(a) structure is the presence of multiply-repeated kringle domains. Cloning of an apo(a) cDNA from a human liver cDNA library by McLean and colleagues in 1987 revealed that apo(a) bears a striking similarity to the serine protease zymogen plasminogen [2]. Indeed, apo(a) contains 10 copies of a kringle motif that is very similar to plasminogen kringle IV, followed by sequences that are highly similar to the kringle V and protease domains of plasminogen. However, because of critical amino acid differences compared to plasminogen, apo(a) is catalytically inactive [3]. Apo(a) is heterogeneous in size, containing a variable number of identically repeated copies of the major repeat kringle domain (kringle IV type 2), which constitutes the molecular basis of the Lp(a) isoform size heterogeneity observed in the population. A general inverse correlation has been described between apo(a) isoform size and plasma Lp(a) levels [4]; this phenomenon arises at the level of Lp(a) production rather than catabolism.

The similarity between apo(a) and plasminogen has generated sustained speculation about the ability of Lp(a) to interfere with the normal fibrinolytic function of plasmin(ogen), which has obvious implications for atherothrombotic disorders. However, definitive identification of the function of Lp(a) either in normal physiology or in vascular disease remains elusive. Indeed, the duality of Lp(a) structure, residing in its similarity to both plasminogen and LDL, implies potential functions of Lp(a) through both proatherosclerotic (based on its similarity to LDL), as well as prothrombotic (based on its similarity to plasminogen) mechanisms. Additionally, unique functions of apo(a) which cannot be attributed to its similarity to plasminogen have been reported, which further complicates our understanding of the role of Lp(a) in atherothrombosis. Our present knowledge as to how Lp(a) is involved in
atherosclerotic/thrombotic disease has been obtained from three main sources: epidemiological studies, in vitro studies of apo(a)/Lp(a), and studies using animal models for Lp(a).

Lp(a) as a Risk Factor for Vascular Disease: Evidence from Clinical Studies

Taken together, the data from epidemiological studies that have been published over the past three decades strongly indicate that Lp(a) is a risk factor for both atherosclerotic and thrombotic diseases. Indeed, elevated plasma Lp(a) concentrations (> 30 mg/dl in most studies) have been identified as a risk factor for peripheral vascular disease, venous thromboembolism, recurrent thrombotic stroke in the pediatric population, and coronary heart disease, the last of which corresponds to the majority of studies [reviewed in 5,6]. In essentially all retrospective case-control studies performed to date, Lp(a) concentrations are elevated in cases compared to controls.

The issue of Lp(a) measurement (see below) as well as issues related to study design and sample storage have served to complicate the interpretation of prospective studies designed to determine the contribution of Lp(a) to future atherosclerotic risk. Indeed, although the balance of prospective studies demonstrate that Lp(a) increases risk for CHD, there are a smaller number of studies which show no effect of Lp(a) on future risk [5]. While the latter studies are in the minority, they have generated controversy over the relative importance of Lp(a) as a risk factor for CHD and its utility as a component of risk assessment.

Controversy has also arisen as to whether Lp(a) is an independent risk factor for CHD. In this regard, several studies have shown that Lp(a) may contribute to CHD risk by potentiating the effects of elevated LDL cholesterol (LDL-C). Interestingly, results from the Bruneck study, which determined the contribution of Lp(a) to both early and advanced carotid atherosclerosis, indicate that while the contribution of elevated Lp(a) to early atherosclerosis is dependent on elevated LDL-C concentrations, the contribution of Lp(a) to advanced atherosclerosis was not [7]. These results may suggest that Lp(a) can play multiple roles in the atherosclerotic process, presumably through different mechanisms.

Some studies over the last decade have sought to define the role of apo(a) isoform size, independent of plasma Lp(a) concentrations, in CHD risk. For example, in the Bruneck study, it was shown that small apo(a) sizes are a risk factor for advanced carotid atherosclerosis, particularly in conjunction with elevated Lp(a) levels [7]. On the other hand, these investigators reported that plasma Lp(a) levels, but not small apo(a) isoform sizes, were predictive of risk for early atherosclerosis and this association was only present when LDL-C levels were also elevated. One study using multiple regression demonstrated that small apo(a) isoform sizes are predictive of risk of angina independently of Lp(a) levels, although other studies failed to detect an independent contribution of apo(a) isoform sizes [8 and references therein]. Since Lp(a) levels and apo(a) isoform sizes are related to each other, resolving this question will require large studies using well-validated assays.

Heterogeneity in Lp(a) size has been problematic in the establishment of Lp(a) measurement methods. Clearly, the use in immunoassays of antibodies that recognize the major repeat kringle of apo(a) (i.e., kringle IV type 2) results in an apo(a) size-dependent bias, with an overestimation of the concentration Lp(a) species larger in size than the calibrator and underestimation of Lp(a) species smaller than the calibrator [9]. Unfortunately, it appears that essentially all commercially-available Lp(a) measurement methods display this bias, with the consequence in many cases of underestimating the predictive power of Lp(a) as a risk factor for
CHD. Interestingly, it has recently been speculated that the use of Lp(a) measurement methods that are dependent on apo(a) size may underlie the lack of association between Lp(a) and CHD risk reported in some earlier prospective studies such as the Physician’s Health Study [10]. This underscores the importance of establishing reliable methods for Lp(a) measurement. Additionally, sufficiently large studies are required in order to address the contribution of apo(a) isoform and LDL-C levels to risk attributable to Lp(a).

**Mechanisms of Lp(a) Pathogenicity**

Insights into possible roles for Lp(a) in vascular disease have been gained through both in vitro studies as well as animal models. Many in vitro studies have probed structure-function relationships between different kringle modules of apo(a) and possible atherosclerotic and/or prothrombotic mechanisms. These studies, which have been greatly facilitated by the use of recombinant variants of apo(a), have helped to delineate unique contributions of specific kringle domains, and suggest that many functions of apo(a) reside within the C-terminal half of the molecule [reviewed in 11]. Analysis of apo(a)/Lp(a) function in vitro has revealed the potential for Lp(a) to inhibit fibrinolysis and to affect the function of arterial smooth muscle cells. A recent study using human vascular endothelial cells has shown that both apo(a) and Lp(a) can elicit cytoskeletal rearrangements [12]. This, in turn, renders the endothelial cells more permeable, which may contribute to a dysfunctional endothelium in vivo. Given that endothelial dysfunction is an early event in atherosclerosis, it is interesting to speculate that consistent with the Bruneck study, Lp(a) levels, in conjunction with elevated LDL levels, may potentiate this process. In this regard, one could envision that the increased permeability of the endothelial cells, mediated by Lp(a), could facilitate the deposition of LDL in the arterial wall. This process would be enhanced by elevated plasma LDL concentrations, which may explain the dependence of Lp(a) risk in early atherosclerosis on concomitant elevations of plasma LDL and Lp(a), but not apo(a) isoform size, as reported in the Bruneck study.

Several animal models have been utilized to probe the function of Lp(a) in a more physiological setting. However, these studies have been generally complicated by the unusual species distribution of this lipoprotein. Indeed, Lp(a) is only present in humans, Old World Monkeys and the hedgehog [13]. This has called into question the applicability of using animals such as mice and rabbits as models for Lp(a) pathogenicity given that the apo(a) gene is not present in these species. However, the balance of opinion is that single and double transgenic animals (i.e., overexpressing both human apo(a) and LDL), can be useful tools to understand the role of Lp(a) in atherosclerosis. Indeed, transgenic apo(a) mouse and rabbit models have been used to study processes such as Lp(a) assembly, structure-function relationships in Lp(a), the role of Lp(a) in atherosclerosis, and the regulation of expression of the apo(a) gene (see Tables 1 and 2).

**Future Directions: Where Do We Go From Here?**

Clearly, our understanding of the physiological and pathological roles of Lp(a) remains incomplete. The most simple hypothesis is that Lp(a) can provide the elusive “link” between the fields of atherosclerosis and thrombosis wherein it can contribute to atherosclerosis through its similarity to LDL while, at the same time, contribute to the persistence of thrombi in the vasculature. However, many studies in vitro and in animal models suggest that the mechanism of
Lp(a) pathogenicity may be much more complicated, and may involve functions of apo(a) that are unique to this moiety such as stimulation of endothelial dysfunction and smooth muscle cell proliferation and calcification.

Both transgenic rabbit and mouse models have their advantages and disadvantages and should be pursued together with ongoing in vitro studies in order to provide insights into the pathogenicity of Lp(a). In theory, these animal models should allow us to directly determine the role of apo(a) isoform size in mediating Lp(a) pathogenicity. In addition, animal models will be an important tool to study therapeutic strategies aimed at lowering Lp(a) or interfering with its harmful effects. The development of an effective method to lower Lp(a) is of critical importance since it would provide a means to prospectively study the effect of lowering plasma Lp(a) concentrations in humans on future risk for vascular disease.

Clinical studies form an important third arm in developing an understanding of the nature and extent to which Lp(a) is a risk factor for vascular disease. Care needs to be taken in the design of these studies to ensure that they are sufficiently powered, and that they utilize methodologies for measuring Lp(a) that are independent of apo(a) isoform size. Additionally, isoform size should be determined where possible in order to develop a better understanding of the relationship between apo(a) isoform size and Lp(a) risk.
Table 1. Mouse models of apo(a)/Lp(a)

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<th>Model</th>
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| YAC apo(a) transgenic mouse (transgene consists of entire human gene including 5'- and 3'-flanking regions) | • estrogen and testosterone ↓ apo(a) mRNA; progesterone had no effect  
• ↓ plasma apo(a) levels following stimulation of the acute phase response | [14]      |
| transgenic apo(a) mouse  
(overexpressing 17K human apo(a) from cDNA) | ↑ atherosclerosis  
no effect on atherosclerosis  
↑ spontaneous atherosclerosis in aged mice  
lack of formation of covalent Lp(a)  
• ↓ plasmin and active TGF-β in arterial wall  
• ↑ osteopontin expression in lesions  
↓ lysis of pulmonary emboli stimulated by tPA | [15-17] [18] [19] [20] [21] [22] |
| double transgenic mouse  
(overexpressing human apo(a) and human apoB) | formation of covalent Lp(a)  
no effect on the extent of lesion formation in LDL-R⁻/⁻ background | [23] [24] |
| transgenic apo(a) mouse  
(overexpressing 17K apo(a) with disrupted LBS in KIV type 10) | • after fat-feeding, lesion area comparable to non-transgenic and ↓↓ than 17K transgenic mice  
• mutant apo(a) not localized in lipid-rich areas, unlike wild-type apo(a) | [25] |
| transgenic human apoB mouse injected with adenoviral vectors encoding apo(a) “mini-gene” or “mini-gene” with disrupted LBS in KIV type 10 | ↓ accumulation of apo(a) in lesions with LBS mutant | [26] |
| transgenic mice overexpressing a four-kringle construct consisting of apo(a) KIV types 5-8 | ↑↑ lesion area after fat-feeding, thus not in agreement with proposed role for strong LBS | [27] |

Abbreviations: YAC: yeast artificial chromosome; 17K: recombinant apo(a) consisting of 17 kringle IV-like repeats (as well as kringle V-like and protease-like domains); TGF-β: transforming growth factor-β; tPA: tissue-type plasminogen activator; LDL-R⁻/⁻: low density lipoprotein receptor knockout; LBS: lysine binding site; KIV: kringle IV
Table 2. Rabbit models of apo(a)/Lp(a)

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| transgenic rabbit overexpressing 17K apo(a) from cDNA                 | covalent Lp(a) formed (~80% efficiency) by interaction of rabbit LDL and human (apo(a))  
  • ↑ diet-induced atherosclerosis  
  • apo(a) deposition associated with intimal smooth muscle cells and ↓ active TGF-β  
  • ↑ markers for immature SMC (i.e. ↑ dedifferentiation)                                                                        | [28]      |
| transgenic rabbit overexpressing 17K apo(a) from cDNA in a WHHL background | covalent Lp(a) formed (~ 20% efficiency)  
  • ↑↑ diet-induced atherosclerosis  
  • ↑ intimal area  
  • more advanced lesions  
  • ↑ calcification of SMC  
  • ↑ matrix Gla protein expression in SMC  
  • ↓ osteopontin expression in SMC  
  • promotion of an osteoblast-like SMC phenotype (↑ osteoblast-specific factor-2 and alkaline phosphatase)  
  ▲ plasma apo(a) concentration, thus suggesting a role for the LDL receptor in clearance of r-Lp(a) in this model | [29]      |
| YAC apo(a) transgenic rabbit (transgene consists of entire human gene including 5’- and 3’-flanking regions) | majority of apo(a) not covalently associated with rabbit LDL, but a high level of non-covalent association observed  
  • apo(a) protein appeared to be truncated | [30]      |
| YAC apo(a) transgenic rabbit co-expressing human apoB from a P1 phagemid | most apo(a) found as part of covalent Lp(a) particle  
  • total apo(a) concentrations not different between animals expressing or not expressing human apoB | [32]      |
| jugular vein thrombolysis model – thrombi formed ex vivo ± 17K apo(a) were injected into rabbit jugular vein | ↓ endogenous and pharmacological (via tPA) thrombolysis in the presence of apo(a) | [33]      |

Abbreviations: TGF-β: transforming growth factor-β; SMC: smooth muscle cells; 17K: recombinant apo(a) consisting of 17 kringle IV-like repeats (as well as kringle V-like and protease-like domains); WHHL: Watanabe heritable hyperlipidemic rabbit; YAC: yeast artificial chromosome
References


