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LIPIDS AND CARDIOVASCULAR DISEASE

Lipoprotein(a) catabolism: a case of multiple receptors

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Summary

Lipoprotein(a) [Lp(a)] is an apolipoprotein B (apoB)-containing plasma lipoprotein similar in structure to low-density lipoprotein (LDL). Lp(a) is more complex than LDL due to the presence of apolipoprotein(a) [apo(a)], a large glycoprotein sharing extensive homology with plasminogen, which confers some unique properties onto Lp(a) particles. ApoB and apo(a) are essential for the assembly and catabolism of Lp(a); however, other proteins associated with the particle may modify its metabolism. Lp(a) specifically carries a cargo of oxidised phospholipids (OxPL) bound to apo(a) which stimulates many proinflammatory pathways in cells of the arterial wall, a key property underlying its pathogenicity and association with cardiovascular disease (CVD). While the liver and kidney are the major tissues implicated in Lp(a) clearance, the pathways for Lp(a) uptake appear to be complex and are still under investigation. Biochemical studies have revealed an exceptional array of receptors that associate with Lp(a) either via its apoB, apo(a), or OxPL components. These receptors fall into five main categories, namely 'classical' lipoprotein receptors, toll-like and scavenger receptors, lectins, and plasminogen receptors. The roles of these receptors have largely been dissected by genetic manipulation in cells or mice, although their relative physiological importance for removal of Lp(a) from the circulation remains unclear. The *LPA* gene encoding apo(a) has an overwhelming effect on Lp(a) levels which precludes any clear associations between potential Lp(a) receptor genes and Lp(a) levels in population studies. Targeted approaches and the selection of unique Lp(a) phenotypes within populations has nevertheless allowed for some associations to be made. Few of the proposed Lp(a) receptors can specifically be manipulated with current drugs and, as such, it is not currently clear whether any of these receptors could provide relevant targets for therapeutic manipulation of Lp(a) levels. This review summarises the current status of knowledge about receptor-mediated pathways for Lp(a) catabolism.

Key words: Lp(a); CVD; LDL; apo(a); apoB; OxPL; LDLR; TLR; scavenger receptors; lectins; plasminogen receptors.

Abbreviations: apo(a), apolipoprotein(a); apoB, apolipoprotein B; ASGPR, asialoglycoprotein receptor; AVS, aortic valve stenosis; CHD, coronary heart disease; CVD, cardiovascular disease; FCR, fractional catabolic rate; GWAS, genome wide association studies; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; Lp(a), lipoprotein(a); LRP, low density lipoprotein receptor-related protein; OxPL, oxidised phospholipids; PCSK9, proprotein convertase subtilisin/kexin type 9; PlgRKT, plasminogen receptor with a C-terminal lysine; SR-B1, scavenger receptor B1; TLR, toll-like receptor; VLDL, very low-density lipoprotein; VLDLR, very low density lipoprotein receptor.

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INTRODUCTION

Lipoprotein(a) [Lp(a)], a plasma lipoprotein first discovered in 1963,[1] as an antigenic variant of low density lipoprotein (LDL), has been at the forefront of research on cardiovascular risk factors for the last decade. Projected into the realm of importance by large genetic studies showing a clear and robust association between *LPA* gene variation, Lp(a) levels, and cardiovascular disease (CVD),[2,3] it is now a well established risk factor. Plasma Lp(a) levels in excess of 50 mg/dL, which occur in 20% of most populations,[4] and to a higher degree in some,[5] constitute a robust risk for the development of CVD.[4] The role of Lp(a) as a risk factor associated with multiple forms of CVD has been well documented for coronary heart disease (CHD),[6,7] stroke,[7,8] and aortic valve stenosis (AVS)[9,10] and is discussed in many recent reviews.[11–13] Its causality for CHD and AVS has been established by Mendelian randomisation studies.[3,14] Most recently, a meta-analysis of data from statin outcome trials has shown that raised Lp(a) levels in statin-treated individuals predict a similarly increased CVD risk to that predicted at baseline.[15] All of this evidence points to a real need for routine Lp(a) measurement and targeted reduction of Lp(a) to reduce CVD events.

The mechanisms by which Lp(a) promotes CVD have been established in numerous *in vitro* and *ex vivo* studies demonstrating its ability to promote inflammation, atherosclerosis, thrombosis and arterial calcification as summarised in recent reviews.[16–18] While there has been some translation of these features in transgenic mice and rabbits, the lack of an appropriate animal model for Lp(a)[18,19] has hindered the translation of these pathogenic properties *in vivo*. However, imaging studies and *ex vivo* analysis of subjects with elevated Lp(a) levels and AVS[20–22] are paving the way for more directly establishing the involvement of Lp(a) in promoting CVD.

With respect to measurement of Lp(a), an initial recommendation that Lp(a) be measured in at-risk patients was made by the European Atherosclerosis Society Consensus

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Panel.[4] European, Canadian and US guidelines for the management of dyslipidaemias now recommend Lp(a) measurement in similar patient subsets.[23] Measurement of Lp(a), however, comes with caveats, as thoroughly discussed in a recent review.[24] The majority of assays used for Lp(a) measurement are immunoassays (commonly ELISAs) that utilise antibodies against the apolipoprotein [apo(a)] component of Lp(a), reporting values as mass protein concentrations. The heterogeneity of apo(a) size [see section on Lp(a) structure] can impose an apo(a) isoform-dependent variation in immunoassay measurement depending on the antibody.[24] Furthermore, the range in size of apo(a) isoforms makes the mass concentration value inaccurate, since the isoform sizes of the reference will rarely be the same as the sample being measured.[24] Ideally, for accurate clinical interpretation, the recommendation is that Lp(a) concentrations be measured with an apo(a) isoform-independent assay that is standardised with a common reference material and reports results as molar concentrations.[24] A recent paper showed that a simple conversion of mass concentration to molar concentration is not appropriate for accurate classification of at-risk patients.[25] Another consideration regarding Lp(a) measurement is that the cholesterol component of Lp(a) is measured as LDL cholesterol.[26] This poses a problem for the interpretation of LDL cholesterol levels with respect to the classification of familial hypercholesterolaemia (FH) patients, since patients with elevated Lp(a) may be falsely classified as FH.[27] On the other hand, an elevated Lp(a) in a true FH patient results in added CVD risk on top of that posed by FH. For these reasons, it is critical that patients suspected of FH be measured for Lp(a) as originally recommended.[4]

Regarding therapies to lower Lp(a), many lipid-lowering drugs (mostly aimed at lowering LDL) have proven useful in reducing Lp(a) levels, as discussed in two recent reviews.[16,28] These include niacin, the apoB secretion inhibitors mipomersen and lomitapide, and the PCSK9 inhibitors (discussed in more detail below). Lipoprotein apheresis, although more cumbersome, has also proven very effective at lowering Lp(a).[16] The recent development of the first specifically targeted therapy for lowering Lp(a) has ignited interest in Lp(a) therapeutics. This comes in the form of apo(a) antisense oligonucleotides that have been shown to significantly reduce apo(a) synthesis and Lp(a) output from the liver[29] and to induce potent reductions in Lp(a) levels.[30] Apo(a) antisense drugs provide a focal point for establishing whether Lp(a) lowering is associated with reduced CVD events, an outcome which should seal the fate of Lp(a) as an important and modifiable CVD risk factor.

Despite many advances in the area of Lp(a) genetics and pathogenicity, several vital questions about the basic biochemistry of Lp(a) still remain, particularly those pertaining to the pathways for its catabolism. A better understanding of the key pathways that remove Lp(a) from circulation could help to better inform the development of future Lp(a)-lowering therapies.

LP(A) STRUCTURE AND ASSEMBLY

Lp(a) (Fig. 1) consists of a LDL molecule complexed to apo(a) via its single copy of apoB.[31] Apo(a) is a large glycoprotein recently evolved from the plasminogen gene within primates to contain two plasminogen kringle domains: kringle IV and kringle V (KIV and KV, respectively).[32] The KIV domain has subsequently evolved into 10 different subtypes through amino acids substitutions, with one of the domains, KIV2, occurring in varying number to produce many different-sized isoforms.[33,34] Apo(a) is synthesised and secreted from the liver.[35] The synthesis of apo(a) is an important determinant of Lp(a) levels, a fact reiterated by the marked Lp(a)-lowering effect of the apo(a) antisense oligonucleotides.[30] After secretion, apo(a) initially binds to apoB lysine residues on LDL via its lysine-binding sites in KIV7 and KIV8[36,37] with different apoB lysine residues being implicated.[38,39] This interaction facilitates the subsequent formation of a disulphide bond between apo(a) Cys4057 of KIV9[40] and apoB Cys4326.[41,42]

The site of Lp(a) assembly is not exactly known. Since LDL is a catabolic product of very low-density lipoprotein (VLDL) which is produced in the circulation, it was hypothesised that the binding of apo(a) to LDL would also occur in the circulation. There is much evidence that an extracellular assembly of Lp(a) can occur in the circulation or on the hepatocyte surface, as studied in mice,[43] cell culture[44–46] and *in vivo* kinetic studies in humans.[47] However,

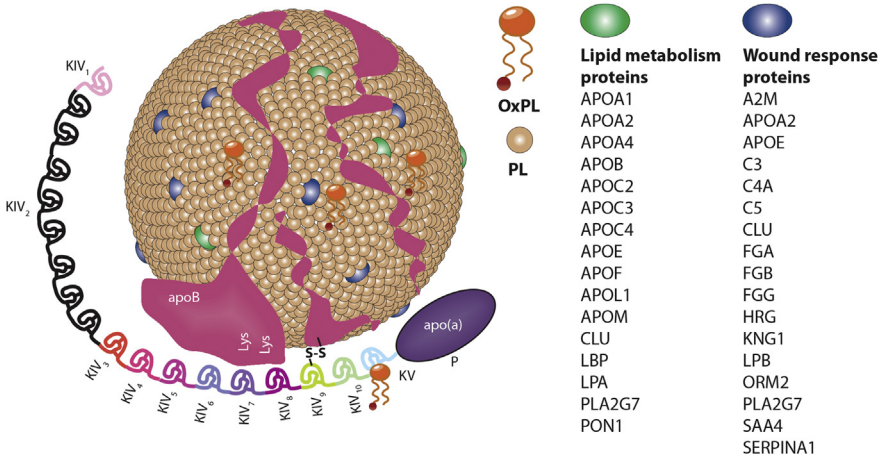

Fig. 1 Lp(a) structure. Lp(a) consists of an LDL molecule attached to apo(a) via a disulphide link to apoB. Oxidised phospholipids (OxPL) are found bound to the apo(a) KV domain and contained in the phospholipid (PL) monolayer on the LDL surface. A number of lipid metabolism proteins (green) are associated with the LDL particle, as are many wound-healing proteins (blue), some of which may be bound to apo(a). The proteins are designated by their gene names. The Lp(a) particle in this figure is modified from that first published in Albers *et al.*[100]

evidence for an intracellular assembly of Lp(a) has also been reported in cell culture[48,49] and human studies.[50,51]

Whether LDL is the only apoB-containing lipoprotein that is capable of binding apo(a) is not certain. There is some evidence that apo(a) can associate with triglyceride-rich lipoproteins, particularly after high-fat feeding in humans[52] and in hypertriglyceridaemic subjects.[53,54] Studies of apo(a) transgenic mice infused with either human VLDL or LDL, however, show that apo(a) preferentially associates with LDL.[43] Biophysical studies have shown that the apoB protein displays different dynamics on the surface of VLDL compared to LDL.[55] A study utilising apoB monoclonal antibodies demonstrated a different exposure of apoB surface residues between VLDL and LDL, particularly in the region near Cys4326.[56] These properties likely underlie the observed different affinity of apo(a) for VLDL and LDL.

Exactly how the disulphide bond between apo(a) and apoB is formed is a mystery. It presumably occurs either via spontaneous oxidation or a facilitated reaction involving isomerase activity as indicated in a previous cell culture study.[57] Also uncertain is the stability of the disulphide linkage in plasma. Some kinetic studies have identified two pools of apo(a) in the circulation[54,58] supporting the hypothesis that there is recycling of apo(a) between circulating lipoprotein particles; however, this would require a reversible cleavage of the disulphide bond.

While apo(a) and apoB constitute the main protein components of Lp(a), a proteomic assessment of purified Lp(a) particles has detected a number of other apoproteins as well as a unique combination of proteins involved in inflammation and wound healing[59] (Fig. 1). Interestingly, Lp(a) contains two phospholipase enzymes: lipoprotein-associated phospholipase A2 (also known as platelet-activating factor acetylhydrolase)[60] and the lysophospholipase D enzyme, autotaxin.[61] Phospholipase A2 enzymes promote the hydrolysis of fatty acids from the *sn*-2 position of glycerophospholipids to produce free fatty acids and lysophospholipids such as lysophosphatidylcholine. Autotaxin acts on lysophosphatidylcholine to release lysophosphatidic acid and choline. Both enzymes are associated with increased CVD risk through their ability to release oxidised fatty acids (lipoprotein-associated phospholipase A2) and lysophosphatidic acid (autotaxin) which are known to stimulate many different inflammatory pathways. This is of interest given that Lp(a) specifically carries a cargo of oxidised phospholipids (OxPL) rich in lysophosphatidylcholine[62] which would generate both oxidised fatty acids and lysophosphatidic acid in the presence of lipoprotein-associated phospholipase A2 and autotaxin. Indeed, two recent studies have documented that the autotaxin-facilitated release of lysophosphatidic acid from Lp(a) OxPL stimulates inflammation and mineralisation in aortic valves, promoting calcification via the nuclear factor kappaB/interleukin 6/bone morphogenetic protein pathway.[61,63]

Where Lp(a) acquires its associated wound healing proteins, or indeed the OxPL, is unknown, although it is presumed that they are taken up by the particle via its interactions with cell surfaces and at sites of inflammation and injury in the body. The apo(a), apoB, and OxPL components all play an important role in Lp(a) catabolism, acting as ligands for various receptors. Whether any of the minor protein components also take part in Lp(a) catabolism is unknown and requires further investigation. There is evidence that apoE may play a role in Lp(a) catabolism, and that some of the proteins found on Lp(a) may act as ligands for some of the putative Lp(a) receptors discussed below.

LP(A) CATABOLISM

An obvious approach for reducing Lp(a) levels is to increase its catabolism, as has been successfully achieved for LDL via the statins which upregulate the LDL receptor (LDLR).[64] However, in contrast to LDL, for which Nobel prize-winning research has clearly delineated its catabolism by the LDLR,[65] the clearance route for Lp(a) is not well defined. Indeed, there appears to be a multitude of proposed pathways for the catabolism of Lp(a), although those genuinely important for clearance from the circulation have not been established. Studies in mice show that the liver provides the main route of clearance of Lp(a) from the circulation with some clearance via the kidney.[66,67] Various receptors in different cell types have been investigated for their role in Lp(a) catabolism. Table 1 summarises the various receptors that have been studied to date for which evidence for a role in Lp(a) catabolism exists. From these studies, five key classes of receptors have emerged with evidence for a role in the uptake of Lp(a), namely 'classical' lipoprotein receptors, scavenger receptors, toll-like receptors, carbohydrate receptors or lectins, and plasminogen receptors (Fig. 2). Some are implicated in uptake of the holoparticle and others in the uptake of specific components, most notably the OxPLs.

LIPOPROTEIN RECEPTORS

The LDLR family, members of which are involved in the holoparticle uptake of chylomicrons, VLDL and LDL, have received the most attention as candidate Lp(a) receptors. The similarity of Lp(a) to LDL makes the LDLR itself an obvious candidate and much effort has been directed at investigating the role of this receptor in Lp(a) clearance. Conflicting data on its involvement, however, make it difficult to conclude a definite role for the LDLR. Evidence supporting a role for the LDLR initially came from studies showing that Lp(a) could bind to human fibroblasts[68–70] via the LDLR. Subsequently, Lp(a) was also shown to bind with high affinity to the human hepatoma cell line, HepG2, via the LDLR,[71] a finding that has been reiterated in subsequent studies investigating the role of proprotein convertase subtilisin/kexin type 9 (PCSK9) in Lp(a) catabolism.[72,73] A seminal study showed similar binding characteristics of Lp(a) and LDL in fibroblasts obtained from wildtype or FH individuals containing mutations in the *LDLR* gene.[70] Furthermore, Lp(a) and LDL displayed similar clearance characteristics *in vivo*, with both lipoproteins showing a delayed clearance in FH subjects.[70] More evidence for the LDLR being important for Lp(a) catabolism came from studies in mice overexpressing the LDLR, which showed a much enhanced Lp(a) clearance.[74] Further *in vivo* support arose from a large study of related FH subjects who had higher Lp(a) levels than non-FH subjects, a result which was also apparent in sib pairs with the same apo(a) alleles.[75] Similarly, results from a large Spanish cohort show heterozygous FH individuals to have significantly higher Lp(a) levels than related individuals without FH.[76]

Despite all of the evidence supporting a role for LDLR as a major receptor for Lp(a), there are cell culture, mouse and human studies that do not support a role for the LDLR in

Table 1 Receptors showing some evidence for acting as Lp(a) receptors

Receptor	Gene	Tissue expression, cell and animal models used	Lp(a) ligands
Annexin A2	*ANXA2*	Ubiquitous, highest in bone marrow and immune, lung, liver and gallbladder, gastrointestinal tract, kidney and urinary bladder, male and female reproductive tissues, adipose and soft tissue, skin. **Cells: human endothelial**	Apo(a)
ASGR1	*ASGR1*	Liver and gallbladder. **Cells: human hepatocytes, monkey fibroblast-like. Animals: KO mice**	Apo(a), orosomuscoid
CD36	*CD36*	Ubiquitous, highest in bone marrow and immune, muscle, adipose and soft tissue. **Cells: murine macrophages**	OxPL
LDLR	*LDLR*	Ubiquitous, highest in male reproductive tissue, medium in endocrine, lung, liver and gallbladder, gastrointesinal tract, kidney and urinary bladder. **Cells: human fibroblasts, human hepatocytes. Animals: KO mice, TG mice, humans**	ApoB, apoE, apo(a)
Galectin-1	*LGALS1*	Ubiquitous, highest in muscle, female reproductive tissue, adipose and soft tissue. **Cell: human endothelial**	Apo(a)
LRP1	*LRP1*	Ubiquitous, medium levels in brain, lung, liver and gallbladder, male and female reproductive tissues, adipose and soft tissue. **Cells: human fibroblasts**	ApoE, alpha2 macroglobulin
Megalin	*LRP2*	Kidney and endocrine. **Cells: murine embyronic yolk sac**	ApoB
PlgRKT	*PLGRKT*	Ubiquitous, highest in bone marrow and immune, lung, gastrointestinal tract, male and female reproductive tissues, skin. **Cells: human hepatocytes, human fibroblasts**	Apo(a)
SR-B1	*SCARB1*	Ubiquitous, highest in endocrine and male reproductive tissues, medium in lung and female reproductive levels. **Cells: human hepatocytes. Animals: KO mice, TG mice, humans**	OxPL
TLR2	*TLR2*	Bone marrow and immune, lung, adipose and soft tissue. **Cells: macrophage. Animals: humans**[a]	OxPL
TLR6	*TLR6*	Bone marrow and immune cells, liver and gallbladder, kidney and urinary bladder. **Cells: macrophages**	OxPL
VLDLR	*VLDLR*	Ubiquitous, medium levels in brain, endocrine, bone marrow and immune, muscle, lung, gastrointestinal tract, kidney and urinary bladder, male and female reproductive tissues, skin. **Cells: murine fibroblasts. Animals: KO mice**	ApoE, apo(a)

Tissue expression data are from human protein atlas (https://www.proteinatlas.org/) and given at the protein level, apart from TLR2 and TLR6, which is at the RNA level. Cell lines and animal models in which the receptors have been studied are in bold.
KO, knockout; TG, transgenic.
[a] Genome wide association studies only.

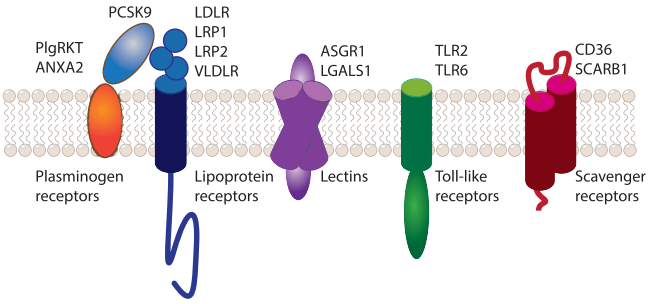

Fig. 2 Candidate Lp(a) receptors. Receptors for which experimental evidence supports their facilitation of Lp(a) uptake or Lp(a) binding are shown. Also shown is the protease PCSK9, which is known to regulate levels of the lipoprotein receptors and interact with annexin A2. The proteins are designated by their gene names.

internalising Lp(a). For example, mouse fibroblasts did not show significant binding of Lp(a) to the LDLR.[77] A study of LDLR knockout mice showed a similar clearance of Lp(a) to that seen in wildtype animals.[66] Furthermore, catabolic studies in FH individuals showed similar clearance rates of Lp(a) between affected subjects and controls,[78–80] with one study suggesting that the LDL from Lp(a) is only cleared after the apo(a) component is released from the particle.[80] These studies are in agreement with family studies showing no difference in Lp(a) levels between FH individuals and non-FH individuals with the same sized apo(a) isoforms.[81]

In line with data from biochemical studies, pharmacological investigation with drugs that increase the level of LDLR expression on the cell surface also show conflicting results regarding their effect on Lp(a) levels. The statin drugs which inhibit 3-hydroxy-3-methyglutaryl coenzyme A reductase and increase *LDLR* gene expression, do not lower Lp(a) levels.[82,83] Indeed, a systematic review of clinical trials showed statins to increase levels of both Lp(a) and OxPL,[84] thus seemingly precluding a role for the LDLR in Lp(a) clearance *in vivo*. On the other hand, the recently approved PCSK9 inhibitors, which increase LDLR levels by inhibiting its degradation, have been shown to significantly lower Lp(a) levels up to 30%[85–87] with the extent of Lp(a) lowering correlating with LDL reduction.[85,87] The mechanism underlying this effect has recently been the subject of intense investigation. *In vitro* experiments in cultured liver cells have shown that PCSK9 inhibitors reduce apo(a) production,[88] an effect reproduced *in vivo* in both monkey[89] and human kinetic experiments.[90,91] There is also evidence that PCSK9 inhibitors can enhance Lp(a) catabolism by increasing the abundance of the LDLR,[72] an effect shown in humans,[91] and more prominently in combination therapy with statins.[90]

New insight into the potential role of the LDLR in Lp(a) uptake has been gained by expression of recombinant PCSK9 in cultured liver cell lines including primary cells. In the presence of low LDL levels induced by statins, Lp(a) internalisation was found to be dependent on PCSK9, implicating the LDLR as a route for Lp(a) catabolism.[73] It was speculated from this that Lp(a) may become a competitive ligand for the LDLR once LDL levels are sufficiently low, a result which may explain some of the controversial results described previously. Furthermore, this appears to be the case *in vivo* as suggested by a recent kinetic study showing that PCSK9

inhibition increases the fractional catabolic rate (FCR) of Lp(a) only in combination with statin treatment.[90] It has been suggested that Lp(a) binds to the LDLR with a lower binding affinity than LDL due to apo(a) masking the LDLR binding site of apoB.[80] Interestingly, the uptake of recombinant apo(a) isoforms by HepG2 is reduced in the presence of PCSK9.[73] Although this could be explained by the presence of another PCSK9-dependent receptor that binds to apo(a), it hints at the possibility of the LDLR binding apo(a), which is intriguing given that the closely related very low-density lipoprotein receptor (VLDLR) is known to interact with apo(a).[92]

Other members of the LDLR family including the VLDLR, LDL receptor-related protein 1 (LRP1) and the LDL receptor-related protein 2 (LRP2, also known as megalin) have been shown to play a role in Lp(a) uptake. The VLDLR is expressed in skeletal muscle, brain, heart, adipose tissue, and in macrophages and endothelial cells. It plays a major role in VLDL clearance via its interaction with apoE,[93] and in concert with LDL receptor-related protein 8 (LRP8, also known as ApoE receptor type 2), functions as a signalling receptor for brain cortex development via an interaction with clusterin.[94,95] The VLDLR was shown to bind Lp(a) and facilitate its uptake in mouse fibroblasts.[92] Furthermore, VLDLR-deficient mice showed a delayed clearance of Lp(a).[92] Competition assays suggested that it was apo(a) rather than LDL that interacted with the VLDLR.[92] Lp(a) uptake via the VLDLR may be of particular relevance for foam cell formation and uptake into endothelial cells;[92] however, the receptor is not expressed in the liver, making it an unlikely candidate for a significant role in Lp(a) clearance.

Like the VLDLR, LRP1 is widely expressed and has many unrelated ligands.[93] However, LRP1 is highly expressed in the liver where it plays a key role in chylomicron-remnant metabolism.[96,97] Lp(a) binds to LRP1 in human fibroblasts,[77,98] with LDL and plasminogen both being able to compete with Lp(a) binding[98] implying apoB and apo(a) as ligands. Like the VLDLR, LRP1 also utilises apoE as ligand for lipoprotein internalisation, unlike the LDLR which utilises apoB. Lp(a), similar to LDL, contains a very low molar ratio of apoE to apoB[99] making it unlikely that apoE is responsible for the interaction of Lp(a) with LRP1. However, Lp(a) does contain significant amounts of alpha2 macroglobulin[59] which is a prominent ligand for LRP1 and has been shown to compete for Lp(a) uptake.[98]

The LRP2 protein is a multi-liganded receptor which plays a role in the uptake of various nutrients and proteins influencing multiple cell signalling pathways. Although not expressed in the liver, it is highly expressed in the kidney, a site that has been implicated as a major site of Lp(a) clearance by individuals with renal disease.[100] Lp(a) was demonstrated to bind to LRP2 in a mouse embryonic yolk sac cell line[101] with apoB as the ligand. Lastly, LRP8 has been investigated as a receptor for Lp(a) due to its similarity to the LDLR and ability to be degraded by PCSK9,[73] although there is no direct evidence for this receptor being involved in Lp(a) catabolism. Interestingly, all of the aforementioned LDLR family members are degraded by PCSK9[102] which is relevant given the effect of PCSK9 on Lp(a) levels. In relation to this, a comprehensive study looking at the effect of PCSK9 on Lp(a) uptake in multiple liver cell lines failed to show a role for LRP1, LRP8, or the VLDLR in Lp(a) internalisation, while reinforcing the role of the LDLR.[73] Interestingly, Lp(a) is reported to bind to PCSK9,[103] a result of yet unknown biological significance.

TOLL-LIKE AND SCAVENGER RECEPTORS

The ability of Lp(a) to promote inflammation *in vitro* via the activation of multiple pathways has been extensively reviewed[16–18] and more recently has been borne out in clinical studies.[20,21] Toll-like receptor (TLR) proteins are expressed by activated immune cells and utilise various oxidised lipid components of lipoproteins as their ligands to activate MAPKs and nuclear factor kappa B pathways to promote cytokine release and inflammation.[104] These pathways are pertinent to Lp(a) which contains significant amounts of OxPL bound to both its LDL and apo(a) components.[62] TLRs often work in conjunction with the scavenger receptor, CD36, as coreceptors (in the case of the TLR4/TLR6 heterodimer)[105] or as ligand sensors (in the case of the TLR2/TLR6 heterodimer).[106] A novel study in macrophages showed that the TLR2/TLR6 heterodimer acts as a receptor for the OxPL component on apo(a) stimulating the cells to undergo apoptosis via ERK activation of NADPH oxidase,[107] a response dependent on CD36.[107] Interestingly, TLR2 was the only receptor significantly associated with Lp(a) levels in a recent large genome wide association study (GWAS).[108] The basis for this association is unclear, given that neither TLR6 or CD36 showed any association. This may indicate an additional role for TLR2 in Lp(a) metabolism beyond its canonical heterodimeric interaction with TLR6 and CD36. Interestingly, activation of TLR2 results in a significant increase in PCSK9,[109] known to be a regulator of Lp(a) levels as discussed above. The CD36 and TLR2 receptors have both been shown to be crucial for the ability of Lp(a) to stimulate IL-8 production in macrophage cell lines via the OxPL moiety bound to apo(a).[110]

The CD36 receptor features much in common with another scavenger receptor, scavenger receptor B1 (SR-B1), for which evidence for a role in Lp(a) uptake is mounting. Widely established as the canonical receptor for high density lipoprotein (HDL) through its ability to facilitate selective uptake of cholesteryl esters from HDL,[111] SR-B1 has also been shown to mediate uptake of VLDL, LDL and the HDL holoparticle.[112,113] Transgenic mice over-expressing SR-B1 in the liver demonstrate enhanced uptake of Lp(a), while SR-B1 knockout mice demonstrate reduced uptake, adding SR-B1 to the repertoire of Lp(a) receptors.[114] Strengthening these data is another study showing that SR-B1 mediates the uptake of OxPL from Lp(a) in HepG2 liver cells promoting the upregulation of the LXR/RXR/ABCA1 pathway.[115] Unlike TLRs and CD36 which largely mediate OxPL-mediated signalling from Lp(a) and are only expressed in immune cells, the Lp(a) holoparticle uptake activity of SR-B1 and its expression in the liver make it a more likely candidate for regulating the catabolism of Lp(a). Some evidence for this has recently been presented in individuals with both elevated Lp(a) and HDL levels who carry a mutation in the gene for SR-B1 (*SCARB1*) disrupting its ability to facilitate lipid uptake.[116] Together, these results provide a sound basis for the involvement of SR-B1 in Lp(a) catabolism. However, further mechanistic studies on how SR-B1 might operate across a wide range of Lp(a) levels (and levels of LDL, for which it can also act as a receptor) are indicated.

LECTINS

Two members of the lectin family of carbohydrate-binding proteins have been implicated in Lp(a) uptake, namely asialoglycoprotein receptor 1 (ASGPR1) and galectin-1. The ASGPR1 protein mediates the endocytosis of desialylated plasma glycoproteins and is exclusively expressed in the liver.[117] The apo(a) protein has a significant content of *O*-linked sugars of the galactosyl type, of which around 20% are desialylated.[118] A study of mice lacking ASGPR1 showed a markedly increased half-life and reduced degradation of Lp(a) by the liver when compared to wildtype mice.[67] Removal of sialic acids from Lp(a) greatly enhanced the Lp(a) clearance rate in wildtype mice, while a bolus of asialo-orosomucoide, a known ASGPR1 ligand, reduced the clearance rate. Furthermore, overexpression of recombinant ASGPR1 in COS-7 cells enhanced the binding and degradation of desialyated-Lp(a).[67] Although these studies strongly suggest that apo(a) is the ligand for ASGPR1, it is of interest to note that orosomucoid is also carried on the Lp(a) molecule.[59] Galectin-1 is a galactose-specific lectin which is ubiquitously expressed.[119] One study found that purified galectin-1 in solution could readily bind Lp(a) with equal affinity for the native and desialylated forms.[120] The binding of Lp(a) to galectin-1 on endothelial cells was also demonstrated.[120] A further study showed galectin-1 to bind Lp(a):LDL complexes from plasma,[121] a feature that could contribute to the accumulation of both lipoproteins in the arterial wall.

PLASMINOGEN RECEPTORS

Plasminogen receptors have been implicated in Lp(a) catabolism for some time due to the hypothesis that apo(a) would display similar protein/protein interactions as its homologue. Indeed, apo(a) has been shown to bind to a number of other proteins that also bind plasminogen, including key players in the fibrinolysis cascade such as fibrin, tissue plasminogen activator and tissue factor pathway inhibitor.[18]

Many groups have shown that Lp(a) binds to plasminogen binding sites on a variety of cells including fibroblasts,[71,98] monocytes,[122–124] endothelial cells[124,125] and liver cells.[71] This was largely demonstrated by competitive binding assays with Lp(a) and plasminogen or lysine analogues, since lysine-dependent binding is a hallmark of plasminogen receptor interactions. Hence it was proposed that Lp(a) must bind to one or more plasminogen receptors.[71] The plasminogen receptor family is diverse with members displaying little sequence homology despite being linked by the common functionality of lysine-dependent plasminogen binding.[126]

The 12 members of the plasminogen receptor family are largely ubiquitously expressed and known for their roles in plasminogen activation, inflammation, cell migration and wound healing.[126] Recently, one family member, plasminogen receptor with a C-terminal lysine (PlgRKT), was found to colocalise with Lp(a) and facilitate a significant proportion of Lp(a) uptake in cultured human liver cells as demonstrated by knock-down and overexpression studies.[127] Furthermore, PlgRKT-mediated Lp(a) uptake was shown to also take place in human fibroblast-like cells.[127] The high homology of apo(a) to plasminogen suggests that it is the ligand responsible for the interaction of Lp(a) with PlgRKT. The binding of plasminogen to plasminogen receptors is mediated by its lysine binding domain which interacts with specific lysine residues on the receptor, commonly a C-terminal lysine.[126] The PlgRKT receptor contains a C-terminal lysine (Lys147)[128] which would be predicted to bind to one of the lysine binding domains in apo(a). The lysine binding site in KIV10 is the most likely candidate, since other lysine binding sites in apo(a) are masked when it is bound to LDL.[18] The importance of PlgRKT *in vivo* is yet to be tested. A PlgRKT transgenic mouse has recently been generated which may prove useful for this endeavour.[129]

It is possible that other plasminogen receptors expressed by the liver, aside from PlgRKT, might be involved in Lp(a) metabolism. The S100 calcium-binding protein (S100A10) and annexin A2 are plasminogen receptors which function as heterotetramers to facilitate plasminogen activation.[130] Interestingly, an interaction between Lp(a) and the annexin A2 monomer has been documented,[131] and Lp(a) was shown to be able to compete with plasminogen for plasminogen binding sites in endothelial cells.[125] Furthermore, it is possible that plasminogen receptors could work in conjunction with lipoprotein receptors in the liver. For example, one study documented that annexin A2 was a binding partner and negative regulator of PCSK9, a connection which would impact on the uptake of Lp(a) by the LDLR.[132] Plasminogen receptors are also highly expressed on activated macrophages and may, along with toll-like and scavenger receptors, play a role in the interaction of Lp(a) with these cells.

APO(A) RECYCLING

The fate of the Lp(a) particle after uptake is poorly understood. It was thought that the LDL component would be degraded in the lysosome as described for LDL.[65] Similarly, it was speculated that the apo(a) component would be subject to degradation either in the lysosome or the proteosome.[133] In a recent *in vitro* study, it was found that apo(a) was recycled following uptake in cultured human liver and fibroblast cells.[127] Here it was demonstrated that after internalisation, Lp(a) dissociates into its two components, with LDL being degraded in lysosomes and apo(a) being recycled via the trans-Golgi and recycling endosomes to be resecreted.[127] The resecreted apo(a) appeared to reassemble into Lp(a) in the liver cell culture media, an observation in line with previous studies showing Lp(a) formation upon addition of apo(a) to the media of cultured hepatocytes.[134]

The recycling of apolipoproteins is not unprecedented, as apoE has been shown to be recycled after uptake.[135] The proportion of apo(a) that is recycled subsequent to Lp(a) internalisation was estimated to be 30%.[127] It is possible that the recycled apo(a) may significantly contribute to circulating Lp(a) levels. Indeed, recycled apo(a) may underlie the observation from certain kinetics studies (see below) that there are two pools of Lp(a) in plasma. The recycled apo(a) could either be reassembled into Lp(a) or alternatively cleared by the kidney, as previous studies have reported the excretion of free apo(a) in urine.[136] Both scenarios would create a second Lp(a) pool with a different turnover than the newly synthesised Lp(a). The mode of regulation of apo(a) recycling and its implications for Lp(a) catabolism are yet unknown and clearly need further investigation to establish their significance.

GENOME WIDE ASSOCIATION STUDIES

In the case of LDL, it is well documented that mutations in the *LDLR* gene have a major effect on its circulating levels via many studies on FH individuals and families. Furthermore, lipoprotein-based GWAS have identified the *LDLR* gene as highly associated with LDL levels.[137] So far GWAS have not found any receptor genes to be clearly associated with Lp(a) levels. In a recent large Lp(a)-specific GWAS, the *LPA* and *APOE* genes were the only genes identified to have any significant association with circulating Lp(a) levels.[108] In a more targeted approach within the same study, variation in 21 genes reported to regulate Lp(a), including 14 candidate Lp(a) receptor genes, were studied.[108] Only one of these, *TLR2*, showed any association with Lp(a) levels after accounting for the *LPA* gene effect, with one variant being associated with low Lp(a) levels.[108] The significance of this association is unclear and warrants further investigation.

The association of Lp(a) levels with apoE is of interest, in particular the association of the apoE2 genotype with lower Lp(a) levels, which has been documented in several studies. The mechanism for this association is unknown. It has been proposed that the lower affinity of apoE2-containing LDL for the LDLR would allow Lp(a) to better compete for binding and therefore clearance by the LDLR.[108,138] Another potential effect could be a reduced assembly of Lp(a) from LDL due to the significant association of the apoE2 genotype with lower LDL levels.[139]

It should be noted that detecting gene associations with Lp(a) levels is difficult because of (1) the substantial influence of the *LPA* gene itself, and (2) the large variation and skewed distribution of Lp(a) levels within any given population. The lack of any association of Lp(a) levels with any of the proposed receptors for Lp(a) may indicate that Lp(a) clearance is mediated by multiple receptors with the importance of any one receptor being dependent on physiological, pharmacological and disease settings. If this should be the case, targeting Lp(a) receptors for therapy may require a complex approach.

KINETIC STUDIES

Lp(a) metabolism has been investigated by a number of groups using stable isotope enrichment and kinetic modelling to characterise the production and clearance of Lp(a). A careful appraisal of these studies and their confounding effects, and the resulting models based on the data generated, is given in a recent review[140] and is not discussed in detail here. The kinetic models paint several scenarios with respect to Lp(a) catabolism. The simplest scenario which has been observed is the one Lp(a) pool model which suggests that apo(a) and LDL are associated with each other in the circulation and remain so until catabolised.[47,50] Other experimental models have shown the presence of two Lp(a) pools with different FCRs.[54,58] Explanations as to how two Lp(a) pools might arise involve various hypotheses regarding apo(a) recycling. These include apo(a) recycling from LDL onto newly secreted VLDL particles in the circulation[54] or apo(a) recycling back into the circulation after Lp(a) uptake by the liver,[127] as discussed above. Most kinetic studies do not directly interrogate the receptors involved in Lp(a) catabolism. However, a recent large kinetic study in the setting of statin and PCSK9 combination therapy has allowed for some dissection. The results from this study showed just a single Lp(a) pool with an FCR similar to LDL which, like LDL, showed an enhanced FCR with the combination therapy.[90] These results are in keeping with cell culture studies[73] that show the LDLR to be important for Lp(a) clearance in the setting of combination therapies targeted at increasing the expression of the LDLR.

CONCLUSION

Many studies have attempted to identify receptors that are responsible for Lp(a) catabolism. These investigations have been performed in many different systems, from the controlled *in vitro* setting of culture cells and purified Lp(a), to the more complex setting of animal studies and *in vivo* kinetic investigations in man, with each setting having its own confounders. What has emerged from these studies is that Lp(a) lends its hand as a ligand for many receptors in many different tissues and cell types. Dissecting which of these receptors are important for the *in vivo* clearance of Lp(a) is challenging and hampered by the lack of an ideal animal model to aid in the translation of results from basic studies. It is likely that any receptor that does emerge as being important for Lp(a) clearance will, like the LDLR, have its own context in which it is significant. Unlike LDL catabolism, where multiple therapies targeting one receptor have effectively been used to lower LDL, it is more likely that for Lp(a) it will be a matter of multiple therapies targeting multiple receptors.

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