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Review article

Atherogenecity of lipoprotein(a) and oxidized low density lipoprotein: insight from in vivo studies of arterial wall influx, degradation and efflux

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Abstract

The accumulation of atherogenic lipoproteins in the arterial intima is pathognomonic of atherosclerosis. Modification of LDL by covalent linkage of apo(a) (resulting in the formation of Lp(a)) or oxidation probably enhances its atherogenecity. Although the metabolism of LDL in arterial intima has been rather extensively characterized, little has been known about the interaction of Lp(a) and oxidized LDL (ox-LDL) with the arterial wall. The present paper reviews a series of recent in vivo studies of the interaction of Lp(a) and ox-LDL with the arterial wall. The results have identified several factors that affect the accumulation of Lp(a) and ox-LDL in the arterial intima and have provided fresh insight into unique metabolic characteristics of Lp(a) and ox-LDL that may explain the large atherogenic potential of these modified LDL species.

Keywords: Arterial wall; Atherosclerosis; Efflux; Influx; Lipoprotein

1. Introduction

Atherosclerosis is the major cause of ischemic heart disease. Because accumulation of cholesterol in the inner layers of arteries is pathognomonic of atherosclerosis [1], intensive research efforts have been directed toward providing insights into the biochemistry, physiology and genetics of cholesterol and lipoprotein metabolism. The major fraction of plasma cholesterol occurs in low density lipoprotein (LDL) and elevated plasma levels of LDL cholesterol are causally related to the development of ischemic heart disease [1–4]. Lipoprotein(a) (Lp(a)) and oxidized LDL (ox-LDL) are modified forms of LDL that might also play important roles in the development of atherosclerotic lesions. The atherogenic effects of LDL, Lp(a) and ox-LDL most likely arise from their accumulation in the arterial wall rather than in plasma [1]. However, the metabolism of Lp(a) and ox-LDL and their impact on atherosclerosis are less well understood than that of LDL. The present review summarizes recent in vivo studies that have provided new insight into the interaction of Lp(a) and ox-LDL with the arterial wall.

During the last ~20 years, in vivo studies have been used to define and quantify steps in the arterial wall metabolism of LDL that are presumed to affect lipoprotein accumulation in the arterial intima. To enter the arterial intima, LDL has to cross the luminal endothelial barrier. The intactness of the endothelium presumably plays an essential role in preventing

Abbreviations: Apolipoprotein(a), apo(a); Apolipoprotein B, apo-B; ε-amino-*n*-caproic acid, ε-aca; Lipoprotein(a), Lp(a); Low density lipoprotein, LDL; Normal LDL, N-LDL; Oxidized LDL, ox-LDL; Trichloroacetic acid, TCA; Tyramine cellobiose, TC.

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atherosclerosis. Compromised barrier function of the arterial wall results in a large intimal clearance of LDL (i.e. a large LDL permeability of the arterial wall) and thus a large mass influx of LDL into the intima [5,6]. A large intimal clearance of LDL is associated with increased risk of aortic atherosclerosis in animal models [7–9]. After crossing the endothelium, some of the LDL particles may be retained in the arterial wall, resulting in a high local concentration of LDL in the arterial intima. Many lines of observations suggest that retention of LDL plays an important role in atherogenesis [10]. For instance, a small arterial wall fractional loss of LDL in vivo (i.e. fraction of the LDL arterial wall pool removed from the arterial intima per unit of time either by efflux or by cellular degradation) is associated with an increased risk of atherosclerosis in rabbits [11,12]. Upon entry into the arterial intima, some of the LDL particles are degraded by arterial wall macrophages and endothelial cells [13–15]. The degradation rate of LDL is increased in the areas of the normal rabbit aorta that are susceptible to diet-induced atherosclerosis [16]. It is unknown whether this association reflects a causal relationship or whether increased degradation of LDL in a nonlesioned aorta serves as a protective mechanism to remove excess LDL from the atherosclerosis-susceptible areas. However, at more advanced stages of atherosclerosis, it is quite likely that degradation of large amounts of LDL by macrophages in the arterial wall results in accelerated foam cell formation and thus growth of the atherosclerotic lesion.

1.1. Lp(a) has a unique structure and a high plasma Lp(a) level is associated with increased risk of cardiovascular disease

Lp(a), like LDL, contains cholesterol, phospholipids, cholesterol esters, triglycerides and apolipoprotein B (apo-B) [17]. In addition, Lp(a) contains apolipoprotein(a) (apo(a)), a glycated protein that is attached to apo-B via a disulfide bridge [18,19]. Apo(a) shares structural similarities with plasminogen [20]. However, in contrast to plasminogen, apo(a) is highly polymorphic [21–23]. There are at least 34 different apo(a) isoforms [24]. The protein mass ranges from $\sim$200–600 kDa [25]. The size heterogeneity is mainly determined by variation at the apo(a) gene locus. The apo(a) gene contains a variable number of repeats that encode for a polypeptide structurally similar to kringle 4 in plasminogen [20,22,26] and the apo(a) size variation among individuals is tightly correlated with the number of kringle repeats in the apo(a) gene [27]. The smaller apo(a) isoforms are associated with the highest plasma Lp(a) levels [21,28].

Epidemiological studies have associated high plasma Lp(a) concentrations (and small Lp(a) isoforms) with an increased risk of cardiovascular disease (reviews in [29] and [30]). The idea that Lp(a) plays a causal role in the progression of atherosclerosis has also been supported by the observation that overexpression of human apo(a) in transgenic mice resulted in accelerated formation of atherosclerotic lesions [31]. Nevertheless, the prognostic value of Lp(a) measurements as a means of assessing the risk of cardiovascular disease is still unsettled.

The fact that Lp(a) accumulates in atherosclerotic lesions, as established by immunohistochemical and biochemical studies [32–36], is another important foundation of the concept that Lp(a) plays a causal role in atherosclerosis. There are several different mechanisms by which Lp(a) accumulation in the arterial wall might promote development of atherosclerosis. Because of its similarity to plasminogen, Lp(a) can compete with plasminogen for binding sites on fibrin surfaces, thereby reducing the conversion of plasminogen to plasmin [37]. This may result in delayed lysis of fibrin clots [37] and/or promote the growth of smooth muscle cells [38]; both effects could stimulate the development of atherosclerosis. Also, Lp(a) shares homologies with LDL and may thus contribute to cholesterol deposition in the arterial wall, either extracellularly or within foam cells.

1.2. Oxidation of LDL—a rate-limiting step in atherogenesis?

In 1981, Henriksen et al. reported that incubation of LDL with endothelial cells changed the properties of LDL markedly [39]. This modified LDL was taken up avidly by macrophages, whereas normal LDL is degraded by macrophages only to a limited extent [40]. The LDL alterations involve lipid peroxidation and can be inhibited by antioxidants [41,42]. Oxidation of LDL affects the migration of leucocytes, is cytotoxic and causes foam cell formation [43–47]. Based on these properties and its presence in atherosclerotic lesions [48,49], ox-LDL has been suggested to play an important role in the development of atherosclerosis [45–47].

Early metabolic studies indicated that ox-LDL in plasma had a half-life of only a few minutes after an intravenous injection into rabbits [50]. Because of this finding and because plasma contains a variety of antioxidants, it was presumed that ox-LDL in atherosclerotic lesions was produced locally in the arterial wall after entrance of normal LDL from plasma [45,46]. However, physical and biological characterizations of plasma lipoproteins [51,52] and recent studies with antibodies to epitopes of ox-LDL [53–55] have strongly suggested that ox-LDL is also present in plasma. Interestingly, one study [54] reported that ox-LDL may be eight times more abundant in the plasma of hemodialysis patients, who are prone to atherosclerotic disease [56], than in the plasma of healthy subjects. These

observations underscore the importance of determining whether ox-LDL in plasma provides a source of ox-LDL in the arterial wall.

2. Transfer of LDL, Lp(a) and ox-LDL into arterial intima–inner media

2.1. Similar mechanism for transfer of Lp(a) and LDL into arterial intima–inner media

In 1989, it was reported that Lp(a) binds to plasminogen receptors on the luminal surface of arterial wall endothelial cells [57,58], raising the possibility that receptor binding of Lp(a) plays a role in its accumulation in the arterial wall. Because transfer of LDL into the arterial wall is believed to occur mainly through non-specific macromolecular sieving [6,59], this notion might imply that the mechanisms involved in transport of Lp(a) and LDL into arterial tissue could be markedly different. To study the mechanism of Lp(a) transfer into the arterial wall and to compare the relative rates of transfer of Lp(a) and LDL into arterial intima–inner media, studies have been performed to determine the intimal clearances of human Lp(a) and LDL in the arterial wall of rabbits [60] and humans [61].

Intimal clearances of Lp(a) and LDL in rabbit aortic arch and in human carotid artery were determined by co-injecting human ^{125}I-Lp(a) and ^{131}I-LDL intravenously and measuring the accumulation of radioactivity in the arterial intima–inner media 3 h later (Fig. 1) [60,61]. The assumptions involved in using this approach to assess the rate of unidirectional lipoprotein (in)flux have been discussed extensively elsewhere [9,60]. The positive correlation between the intimal clearances of Lp(a) and LDL in both rabbit and human intima–inner media suggested that similar factors determine the rate at which Lp(a) and LDL are transferred into the arterial wall (Fig. 1). Thus, transfer of Lp(a) into the arterial wall, like that of LDL [6,59], probably occurs mainly through macromolecular sieving. Moreover, the positive correlation between intimal clearances of Lp(a) and LDL could be demonstrated in rabbits with only trace amounts of human Lp(a) in plasma as well as in rabbits with high human Lp(a) plasma levels of 0.33 mg/ml (induced by a bolus injection of $\sim$45 mg of unlabeled human Lp(a) immediately before the determination of the intimal clearance of Lp(a) and LDL) [60]. Also, the intimal clearance of Lp(a) in nonlesioned aortic tissue was similar in rabbits with low and high plasma Lp(a) levels [60,62,63]. These observations suggest that the mass influx of Lp(a) into the arterial wall increases in proportion with the plasma Lp(a) concentration. Moreover, if a large influx of Lp(a) results in increased accumulation of Lp(a) into the arterial wall, these observations might provide a simple mechanistic foundation for the epidemiological association between a high plasma level of Lp(a) and atherosclerotic disease. It should be noted, though, that the plasma Lp(a) concentration varies by a factor of more than a thousand between different human individuals [28] and it cannot be excluded that the influx mechanism may be saturated at very high plasma Lp(a) levels.

When determined simultaneously over 3 h, the intimal clearance of Lp(a) tended to be smaller than the intimal clearance of LDL, both in rabbits and in hu-

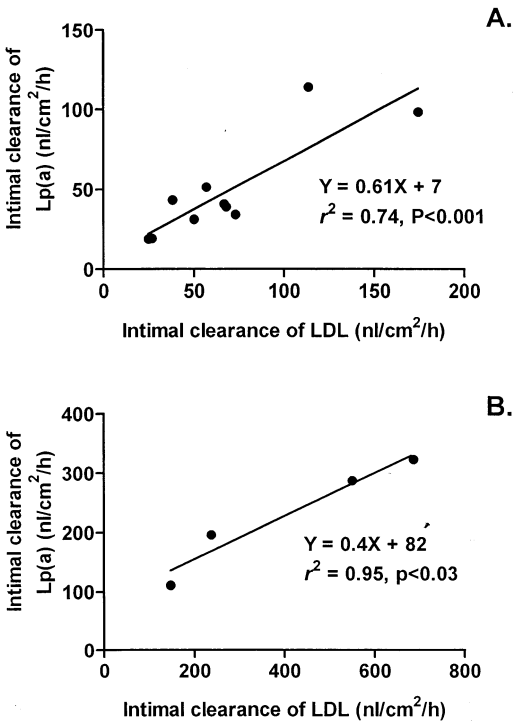

Fig. 1. Simultaneously measured intimal clearance of Lp(a) and LDL in the intima–inner media of rabbit aorta (A) and human carotid artery (B). Each point represents values from one rabbit or one human subject. Human Lp(a) and LDL were labeled with ^{125}I and ^{131}I, respectively and injected intravenously into rabbits ($n=10$) or human subjects scheduled for endarterectomy of a stenosed carotid artery ($n=4$). The rabbits had either received an intravenous injection of $\sim$45 mg of human Lp(a) immediately before injection of labeled lipoproteins ($n=4$) or were fed 1% cholesterol–enriched chow ($n=6$). After 3 h, the intima–inner media of the aortic arch (rabbits) or a segment of the endarterectomy specimen (humans) was isolated and minced. Proteins in tissue homogenates and plasma were precipitated with 15% trichloroacetic acid (TCA) and intimal clearances of Lp(a) and LDL were calculated as the amount of TCA-precipitable radioactivity in the arterial tissue divided by the mean TCA-precipitable plasma radioactivity concentration and the length of the experimental period. In the human studies (B), intimal clearance of ^{125}I-Lp(a) was calculated after correction for contaminating ^{125}I-LDL in plasma (^{125}I-LDL was mainly derived from the conversion of ^{125}I-Lp(a) to ^{125}I-LDL after injection of labeled lipoproteins). The correlation coefficient (r) for the association between intimal clearances of Lp(a) and LDL was calculated by linear regression analysis. The depicted data are adapted from [60] (A) and [61] (B) with permission.

mans [60,61]. Because Lp(a) is larger than LDL, this finding is also in accordance with the idea that molecular sieving is the main mechanism for transport of Lp(a) into the arterial wall [6,64]. This conclusion, nevertheless, was challenged by the observation that the aortic intimal clearance of Lp(a) was higher than that of LDL, when labeled Lp(a) and LDL were coinjected into rabbits 1 h before the aorta was removed [60]. These latter findings may reflect differences in the metabolism of Lp(a) and LDL during the initial 3 h after entrance into the arterial intima–inner media. However, the idea of a pronounced difference in the metabolism of Lp(a) and LDL in the arterial wall of rabbits was not supported by later studies on Lp(a) arterial wall influx and efflux in a larger number of rabbits [62,63]. In those studies of lipoprotein accumulation in the aortic intima–inner media after 3 and 24 h of exposure, there was no indication of a greater intimal clearance of Lp(a) than of LDL (Fig. 2).

The cause of the opposite results on the relative intimal clearances of Lp(a) and LDL after 1- and 3-h exposures of rabbit aorta to the labeled lipoproteins [60] is unclear. It is well known among Lp(a)-investigators that purified Lp(a) can be unstable. Also, Lp(a) can be oxidized [65]. The production time for the batches of purified Lp(a) that were used in the 1-h studies was longer ($\sim$2 weeks) than it was for the batches used in the 3-h studies and later studies (3–8 days). Therefore, it is possible that the Lp(a) used in the 1-h experiments had been slightly oxidized. Because ox-LDL rapidly accumulates in the arterial intima [66], oxidation of Lp(a) used in the 1-h experiment may have led to the larger intimal clearance of Lp(a) than LDL in the initial 1-h experiments.

2.2. Accelerated transfer of Lp(a) into atherosclerotic and balloon injured aortic intima–inner media

Immunohistochemical and biochemical characterizations have indicated that Lp(a) accumulates preferentially in atherosclerotic lesions compared with nonlesioned arterial intima [33,34,36,67]. However, these studies cannot be used to assess whether accumulation of Lp(a) in atherosclerotic tissue results from accelerated influx or decreased efflux. It is well established that an increased intimal clearance of LDL contributes to the accumulation of LDL in atherosclerotic arteries [5,9,68–70]. Thus, based on the idea of a similar mechanism for transfer of Lp(a) and LDL into the arterial wall, one would predict that development of atherosclerosis is accompanied by an increase in the intimal clearance and thus arterial wall influx of Lp(a). To test this hypothesis, a recent study determined the aortic intimal clearance of Lp(a) in rabbits that had been fed cholesterol-enriched chow for $\sim$6 months and had aortic atherosclerosis and in rabbits that had been

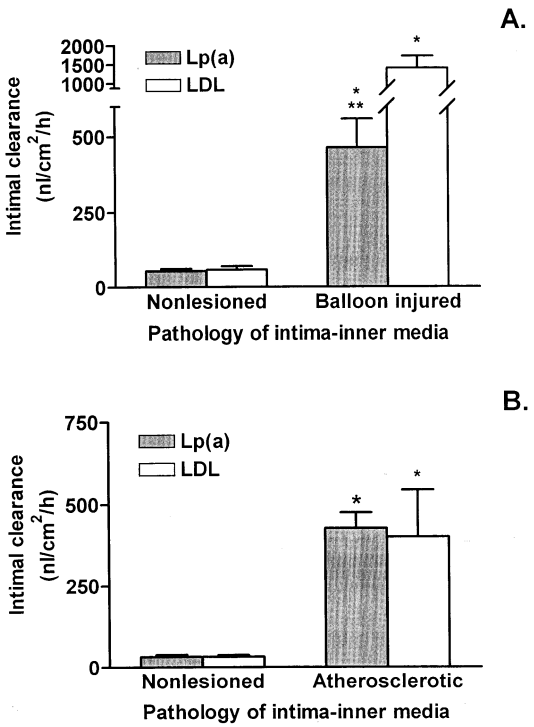

Fig. 2. Intimal clearance of Lp(a) and LDL in the intima–inner media of nonlesioned aortic arch and balloon-injured thoracic aorta (A) and in the intima–inner media of the proximal segment of the aortic arch of nonlesioned and atherosclerotic aortas (B) of rabbits. Human ^{125}I-Lp(a) (or human ^{125}I-LDL) was injected intravenously 26 h and human ^{131}I-Lp(a) (or human ^{131}I-LDL) 3 h, before the aorta was removed. From the amounts of TCA-precipitable radioactivity in plasma and intima–inner media, intimal clearance was calculated using a one-pool-compartment model that allows corrections for loss of labeled lipoproteins from the arterial tissue during the experimental period [62,63]. (A) Intimal clearance in nonlesioned and balloon-injured aortic intima–inner media was determined in rabbits, that had been fed 1% cholesterol for 1 week; balloon injury was inflicted an average 3 days before determination of intimal clearance; Lp(a), $n=8$; LDL, $n=7$. (B) Intimal clearance in nonlesioned and atherosclerotic intima–inner media was determined in rabbits that had been cholesterol-fed for 1 week and $\sim$6 months, respectively; Lp(a), $n=6$ and $n=6$ in the nonlesioned and atherosclerotic group, respectively; LDL, $n=7$ and $n=6$ in the nonlesioned and atherosclerotic group, respectively. *$P=0.0001$ versus nonlesioned intima–inner media. **$P=0.0001$ versus LDL in balloon-injured intima–inner media. Values are means $\pm$ S.E.M. The depicted data are adapted from [62] (A) and [63] (B) with permission.

cholesterol-fed for only 1 week and had no atherosclerosis [63]. In these studies, each rabbit received, by intravenous injection, ^{131}I-Lp(a) (or ^{131}I-LDL) 26 h before and ^{125}I-Lp(a) (or ^{125}I-LDL) 3 h before the aorta was removed. This experimental design allows calculation of the aortic intima clearance of Lp(a) (or LDL) corrected for loss of newly entered labeled Lp(a) from the intima–inner media during the experiment [62,63,69,71,72]. As expected, the intimal clearance of Lp(a), like that of LDL, was markedly greater in atherosclerotic than in nonlesioned intima–inner media (Fig. 2). Moreover, the difference in intimal clearance

of Lp(a) between atherosclerotic and nonlesioned aortas was most pronounced in the aortic arch segments, which had the most extensive atherosclerosis [63]. These results indicate that increased influx of Lp(a) contributes to accelerated accumulation of Lp(a) in atherosclerotic lesions.

An increased influx of Lp(a) may also cause specific accumulation of Lp(a) in balloon-injured arteries. The intimal clearance appeared 8-fold greater in balloon-injured thoracic aorta than in nonlesioned aortic arch of rabbits [62] (Fig. 2). This finding suggests that the endothelium provides a significant barrier for the transfer of Lp(a) into the arterial wall.

Fig. 2 indicates that the intimal clearance of Lp(a) was significantly less than that of LDL in balloon-injured rabbit thoracic aorta. It cannot be excluded that this finding partly reflects a faster entry of LDL than of Lp(a) into balloon-injured arterial wall. However, it is likely that the finding reflects, at least to some extent, a difference in the severity of the inflicted balloon injuries between the rabbits used to study Lp(a) and those used to study LDL. The flux of labeled lipoproteins is faster into severely injured than into mildly injured rabbit aortas [71]. The balloon injuries were more severe in the rabbits used to study LDL than in those used to study Lp(a), as judged from the cholesterol content of the injured intima–inner media and from evaluation of histological sections of the injured aortas [62].

2.3. Oxidation of LDL accelerates its transfer from plasma into aortic intima–inner media

Because recent studies have identified epitopes of ox-LDL in plasma, it has been of interest to determine whether mildly oxidized LDL can circulate in plasma for prolonged periods and the extent to which it enters the arterial wall. Two studies, one in humans [73] and one in normal rabbits [66], have addressed these questions. Iuliano et al. injected ^{99m}technetium-labeled ox-LDL intravenously into human subjects and used a gamma camera to assess accumulation of ox-LDL in the carotid artery wall semiquantitatively. These studies suggested that ox-LDL accumulates in atherosclerotic lesions in preference to nonlesioned arterial sites. To compare plasma and arterial wall metabolism of normal LDL and ox-LDL directly, Juul et al. coinjected mildly oxidized ^{131}I-LDL and normal ^{125}I-LDL intravenously into rabbits and measured the plasma decay and accumulation of the two LDL species in the aortic intima–inner media. Although removed from the plasma more rapidly than normal LDL, ox-LDL circulated in plasma for prolonged periods after the intravenous injection. Remarkably, the accumulation of ox-LDL in the intima–inner media of the thoracic aorta vastly exceeded that of normal LDL 1, 3 and 24 h after the intravenous injection of the labeled LDL species (Fig. 3). Because the accumulation of iodinated LDL in the intima–inner media of rabbits 1 and 3 h after an intravenous injection mainly reflects the unidirectional transfer of LDL from plasma into the arterial wall, these data suggest that ox-LDL in plasma is transferred into the arterial wall in preference to normal LDL.

The mechanism of this preferential transfer of ox-LDL into the arterial wall is unknown. The extent of oxidation of ^{131}I-LDL was closely associated with the excess accumulation of ^{131}I-ox-LDL compared with ^{125}I-labeled normal LDL in the aortic intima–inner media after a 3-h exposure [66]. This finding suggests that oxidation-specific changes in ^{131}I-ox-LDL caused it to accumulate preferentially in the arterial intima–inner

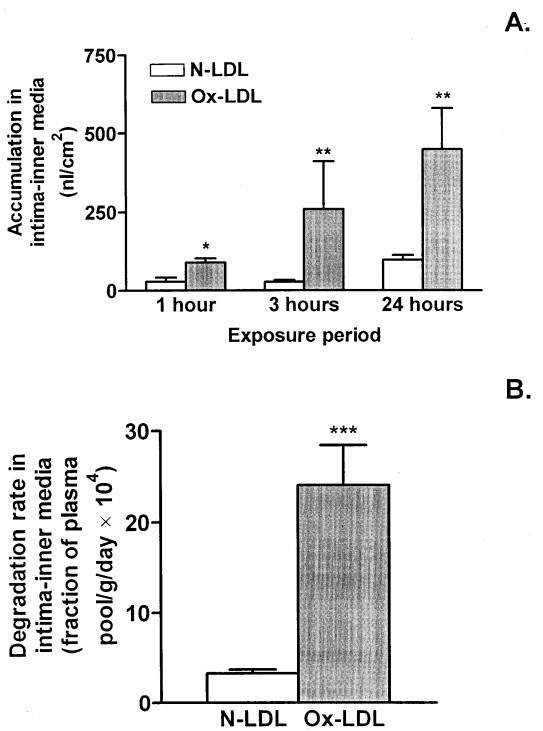

Fig. 3. Accumulation (A) and degradation rate (B) of normal LDL (N-LDL) and ox-LDL in the intima–inner media of the thoracic aorta of rabbits. (A) Human LDL was labeled with ^{125}I (^{125}I-LDL was designated N-LDL) or ^{131}I. ^{131}I-LDL (200 μg protein/ml) was mildly oxidized by incubation with Cu^{2+} (5 μmol/l) at room temperature for 8 h; this LDL was designated ox-LDL. N-LDL and ox-LDL were coinjected intravenously into chow-fed rabbits 1 ($n=6$), 3 ($n=7$), or 24 h ($n=8$) before the aorta was removed. The accumulation of N-LDL and ox-LDL in the intima–inner media was calculated as TCA-precipitable radioactivity in that tissue (cpm/cm^2 surface area) divided by the mean plasma TCA-precipitable radioactivity concentration (cpm/nl). (B) Human LDL was doubly labeled with ^{125}I and ^{131}I-tyramine cellobiose (TC); oxidation of ^{131}I-TC/^{125}I-LDL was performed as described for ^{131}I-LDL. The degradation rates of N-LDL ($n=6$) and ox-LDL ($n=6$) were calculated from intima–inner media and plasma radioactivity concentrations as described. *$P=0.04$, **$P=0.02$ and ***$P=0.005$ vs. N-LDL. Values are means ± S.E.M. The depicted data are adapted from [66] with permission.

media. One possibility is that the increased negative charge of ox-LDL facilitated its passage across the endothelial barrier at microdomains that allow passage of negatively charged macromolecules [74]. Receptor-mediated uptake of ox-LDL at the luminal surface of arterial wall endothelial cells, may also contribute to the increased transfer of ox-LDL into the arterial wall. Oxidation of LDL changes the chemical composition of apo-B [75], reducing its affinity for the LDL receptor, but markedly increasing its affinity for scavenger receptors [45,46,76]. Notably, vascular endothelial cell expression of a receptor for ox-LDL was recently reported [77]. Studies by Sparrow et al. revealed that there is a lag time between scavenger-receptor mediated cellular internalization and subsequent degradation of ox-LDL [78]. Thus, even though ox-LDL may eventually be degraded in the endothelial cells, selective receptor-binding and cellular internalization of ox-LDL may have caused the observed larger accumulation of radiolabeled ox-LDL in the arterial wall.

3. Retention of LDL, Lp(a) and ox-LDL in arterial intima–inner media

3.1. Balloon injury leads to specific retention and accumulation of Lp(a) in aortic intima–inner media

Upon entry into the arterial intima, lipoproteins may be trapped by binding to the extracellular matrix or to cell surfaces. In vitro data suggest that Lp(a) binds to proteoglycans and fibrin with a greater affinity than LDL [79,80]. To test whether this distinct feature of Lp(a) would cause a lower fractional loss of Lp(a) than of LDL from the arterial wall in vivo, a study in rabbits has compared the arterial wall fractional loss of Lp(a) and LDL 3 days after a balloon injury of the thoracic aorta [62]. Balloon injury facilitates deposition of fibrin at the luminal surface of the artery [81] and exposes the subendothelial structures to the blood components. In those studies, ^{131}I-Lp(a) (or ^{131}I-LDL) was injected intravenously 26 h and ^{125}I-Lp(a) (or ^{125}I-LDL) 3 h, before the aorta was removed. Fractional loss calculations were based on a one-pool-compartment model describing the kinetics of newly entered labeled lipoproteins in the arterial wall [62,63,69,71,72].

Several assumptions are implicit in using the one-compartment model to describe the metabolism of lipoproteins in the arterial intima–inner media [62,63,69,71,72]. Most of these assumptions have been discussed and validated in studies of LDL arterial wall metabolism in hypercholesterolemic rabbits and monkeys [12,69,82]. In a recent study, Tozer and Carew determined the concentration of ^{125}I-LDL in the arterial wall of normo- and hypercholesterolemic rabbits at various time points (0.5–72 h) after injection of the labeled LDL. They assessed the mean residence time ($=1$/fractional loss) of LDL in the arterial wall with a stochastic analysis that does not hold the assumptions of a one-compartment model [83]. Interestingly, the stochastic model and a one-compartment model which used the aortic radioactivity accumulation after 1 and 24 h gave similar mean residence times of LDL in the aorta of rabbits that were hypercholesterolemic after 2–3 weeks of cholesterol feeding [83]. However, the one-compartment model tended to underestimate the mean residence time in normocholesterolemic chow-fed rabbits. The studies of LDL and Lp(a) arterial wall fractional loss discussed in this paper were all performed in rabbits that had been cholesterol-fed for ~ 1 week or more. This suggests that the values of fractional loss of LDL [62,63] probably describe the kinetics of LDL in the intima–inner media correctly. Calculations of fractional loss are dependent on the amount of tracer leaving the intima–inner media being proportional to the tracer pool in the intima–inner media. Since rabbits do not have Lp(a), binding sites for newly entered human Lp(a) that are saturated and quantitatively insignificant at physiological plasma Lp(a) levels might exist in the rabbit aorta. Such binding sites could cause an incorrectly small fractional loss of labeled Lp(a). However, the accumulation of labeled Lp(a) in the arterial wall 5–10 min, 3 and 24 h after an intravenous injection of labeled Lp(a) was similar in rabbits that received a bolus injection of unlabeled human Lp(a) prior to the labeled Lp(a) and in rabbits that did not [60,62,84]. Also, the fractional loss of labeled Lp(a) from nonlesioned aortas of cholesterol-fed rabbits appeared unaffected of a bolus injection of unlabeled Lp(a) [62,63]. The one-compartment model of the arterial intima–inner media has not been formally validated for Lp(a) and the absolute values of fractional loss of Lp(a) should be interpreted with caution. However, there are several indications that the observed differences between LDL and Lp(a) fractional losses [62] and between fractional losses of Lp(a) in normal, atherosclerotic and balloon injured aortas probably reflects true biological characteristics of Lp(a) [62,63]. Importantly, when the efflux rates of LDL and Lp(a) were estimated as 'crude fractional loss' which does not depend on the one-compartment model, the overall results and conclusions were similar to those drawn from fractional loss values [63].

The fractional loss of Lp(a) in the balloon-injured segment was 27% of that of LDL, whereas the fractional loss in the nonlesioned segment of the aorta was similar for Lp(a) and LDL [62] (Fig. 4). Furthermore, when labeled Lp(a) and LDL were coinjected into rabbits 23 h before the aorta was removed, the accumulation of Lp(a) exceeded that of LDL in the balloon-injured aortic segment but not in the nonlesioned segment [62]. These observations provide in vivo support for the

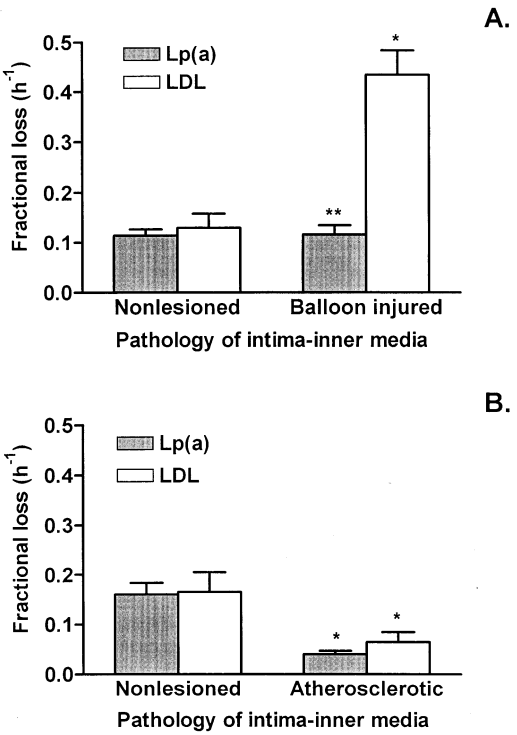

Fig. 4. Fractional losses of Lp(a) and LDL in the intima–inner media of nonlesioned aortic arch and balloon injured thoracic aorta (A) and in the intima–inner media of the proximal segment of the aortic arch of nonlesioned and atherosclerotic aorta (B) of rabbits. Human ^{125}I-Lp(a) (or human ^{125}I-LDL) was injected intravenously 26 h and human ^{131}I-Lp(a) (or human ^{131}I-LDL) 3 h, before the aorta was removed. From the amounts of TCA-precipitable radioactivity in plasma and in the aortic intima–inner media, the fractional loss (i.e. the fraction of the arterial wall pool of labeled lipoproteins that is removed from the arterial wall per hour) was calculated using a one-pool-compartment model. (A) Fractional loss in nonlesioned and balloon-injured aortic intima–inner media was determined in rabbits, that had been fed 1% cholesterol for 1 week; balloon injury was inflicted an average of 3 days before determination of fractional loss; Lp(a), $n=8$; LDL, $n=7$. (B) Fractional loss in nonlesioned and atherosclerotic intima was determined in rabbits that had been cholesterol-fed for 1 week and $\sim$6 months, respectively; Lp(a), $n=6$ and $n=6$ in the nonlesioned and atherosclerotic group, respectively; LDL, $n=7$ and $n=6$ in the nonlesioned and atherosclerotic group, respectively. $^{*}P=0.0001$ versus nonlesioned intima–inner media. $^{**}P=0.0001$ vs. LDL in balloon-injured intima–inner media. Values are means $\pm$ S.E.M. The depicted data are adapted from [62] (A) and [63] (B) with permission.

hypothesis that Lp(a) accumulates selectively compared with LDL in the arterial wall at sites of endothelial injury.

To assess the mechanism for the smaller fractional loss of Lp(a) than of LDL in balloon-injured aorta, the balloon-injured intima–inner media was minced and washed three times with phosphate-buffered saline to remove loosely bound labeled Lp(a) and LDL [62]. The amount of tightly bound Lp(a) exceeded that of LDL in balloon-injured arteries that had been exposed to the labeled lipoproteins for 23 h, supporting the idea of selective binding of Lp(a) to components of the injured intima–inner media [62].

The lysine-dependent association of Lp(a) with fibrin has been proposed to be an important mechanism of Lp(a) accumulation in atherosclerotic lesions [85]. To evaluate whether tightly bound Lp(a) might adhere to the balloon-injured aortic intima–inner media in a lysine-dependent manner, the arterial tissues were washed with a buffer containing ϵ-amino-*n*-caproic acid (ϵ-aca), a lysine analogue that competes with Lp(a) for binding to lysine [26,37]. Surprisingly, similar and only small amounts of tightly bound labeled Lp(a) and LDL could be extracted from the balloon-injured intima–inner media with ϵ-aca [62]. Thus, the idea that the smaller fractional loss of Lp(a) than of LDL from the balloon-injured aorta reflects preferential binding of Lp(a) to lysine residues on fibrin within the arterial wall could not be supported.

Lp(a) can be released from human arterial tissues obtained at autopsy by digestion with plasmin [67]. This indicates that Lp(a) can be incorporated into fibrin clots in the arterial wall. Therefore, the smaller fractional loss of labeled Lp(a) than of LDL from balloon-injured aortas might reflect embedding of Lp(a) into fibrin clots formed at the luminal surface of the injured vessel. Interestingly, fibrin is continuously deposited in the arterial wall from 6 h to at least 2 weeks after a balloon injury of the carotid artery in rabbits [86] and a recent study demonstrated abundant accumulation of Lp(a) in the iliac artery of monkeys 2 weeks after balloon injury [87].

Balloon injury of the rabbit aorta is accompanied by accumulation of glycosaminoglycans in the injured vessel [88]. Preferential binding of Lp(a) to glycosaminoglycans [79] may have reduced the fractional loss of Lp(a) compared with LDL in balloon-injured aorta. Binding of Lp(a) to other extracellular matrix components such as fibronectin may also be involved [89,90]. Interestingly, Lp(a) binds a matrix synthesized by aortic endothelial cell in vitro with increased affinity after heparinase treatment of the matrix and this increased binding can be reduced 60% by anti-fibronectin antibodies [91].

3.2. Retention of Lp(a) and LDL in atherosclerotic intima–inner media

To investigate whether retention of Lp(a) might contribute to Lp(a) accumulation in atherosclerotic lesions, the fractional losses of Lp(a) have been determined in rabbits with nonlesioned aortas and in rabbits with atherosclerosis. Fractional loss was determined with an one-pool-compartment model and the experimental design was similar to that used to study fractional loss of Lp(a) and LDL in balloon-injured aortas (see discussion above) [62,63]. The fractional loss of Lp(a) in

atherosclerotic intima–inner media was on average 25% of that in nonlesioned intima–inner media, suggesting that retention of Lp(a) is an important mechanism that may cause accumulation of this lipoprotein particle in atherosclerotic lesions [63] (Fig. 4). In the same study, the fractional loss of LDL was also smaller in atherosclerotic than in nonlesioned intima–inner media (Fig. 4). A later study has used a stochastic model, which does not hold the assumptions of the one-pool-compartment model, to compare the mean residence times of LDL in atherosclerotic and nonlesioned rabbit aortas [83]. That study further supported the notion that the mean residence time of LDL is longer in atherosclerotic than in nonlesioned aortas of cholesterol-fed rabbits. Taken together, these studies provide strong evidence to support the hypothesis that retention of Lp(a) and LDL promotes the accumulation of these plasma lipoprotein species in atherosclerotic lesions.

A previous study indicated that Lp(a) may accumulate to a greater extent than LDL in veins used as coronary bypass grafts [35]. Also, autoradiographic studies of mouse aortas after an intravenous injection of radiolabeled Lp(a) or radiolabeled LDL suggested that Lp(a) accumulates faster than LDL in the arterial wall [92]. However, a study of rabbit aortas found no significant difference in accumulation or fractional loss between Lp(a) and LDL in nonlesioned or atherosclerotic arteries [63] (Fig. 4). In a statistical analysis of fractional losses of Lp(a) and LDL in six segments of nonlesioned and atherosclerotic intima–inner media combined, the fractional loss of Lp(a) was on average 73% of that of LDL. This effect of lipoprotein type was of borderline statistical significance (95% confidence interval: 51–104%, $P = 0.07$) [63]. There are several possible explanations for these discrepant findings. Although differences in experimental procedures may explain the different findings of the studies in mice and rabbits [63,92], the marked structural differences between the experimentally induced fatty streak lesions in cholesterol-fed rabbits and the advanced lesions in coronary bypass grafts in humans [1] may affect the relative accumulation of Lp(a) and LDL in these two types of arterial tissue. The lesions in cholesterol-fed rabbits predominantly are fatty streaks composed of macrophage-derived foam cells and only small amounts of extracellular matrix deposits, whereas complex vascular lesions in humans are often characterized by a massive accumulation of extracellular matrix components as well as fibrin deposits [1]. Conceivably, Lp(a) may bind more avidly than LDL to components of advanced human lesions that are absent or only present in small amounts in fatty-streak lesions of cholesterol-fed rabbits. Thus, observations on arterial wall metabolism of lipoproteins in small animal models, such as rabbits and mice, should be extrapolated to humans with utmost caution.

3.3. Retention of ox-LDL in aortic intima–inner media

No detailed quantitative analysis on the relative fractional losses of ox-LDL and normal LDL from the arterial intima in vivo is available. Using average values of plasma radioactivity decay and accumulation of radiolabeled ox-LDL and normal LDL in nonlesioned rabbit aorta 3 and 24 h after intravenous injection of labeled LDL, Juul et al. estimated that the fractional loss of ox-LDL is similar to that of normal LDL in nonlesioned intima–inner media [66]. This finding is in accordance with data from Chang et al., who studied the accumulation of radiolabeled ox-LDL in rabbit aorta after balloon injury and concluded that the specific pattern of accumulation of ox-LDL in the healing aorta was independent of oxidation-specific changes in the LDL particle [93]. Moreover, ox-LDL binds to arterial wall proteoglycans with less affinity than normal LDL in vitro (Dr G. Camejo, University of Göteborg, Sweden, personal communication). Thus, the idea that enhanced accumulation of ox-LDL compared with normal LDL in the arterial wall is caused by retention of ox-LDL cannot be supported.

4. Cellular degradation of LDL, Lp(a), and ox-LDL in arterial intima–inner media

LDL is degraded in both nonlesioned and atherosclerotic arterial intima [13–15]. Physiologically, uptake and degradation of LDL by endothelial and smooth muscle cells in nonlesioned arteries may conceivably provide cholesterol for the maintenance of cell membranes. In contrast, uptake and degradation of LDL by macrophage-derived cells in atherosclerotic lesions may serve as a protective mechanism to remove excess LDL particles from the intima, which under some (unknown) circumstances may lead to foam cell formation.

4.1. Lower degradation rates of Lp(a) than of LDL in nonlesioned intima–inner media

To compare degradation rates of Lp(a) and LDL in aortic intima–inner media, Lp(a) (or LDL) has been doubly labeled with both ^{125}I and ^{131}I-tyramine cellobiose (TC) and coinjected intravenously into rabbits 24 h before the aorta was removed [84]. Upon cellular degradation of labeled Lp(a) (or LDL) in a given tissue, ^{125}I diffuses out of the cells and leaves the tissue, while ^{131}I-TC remains trapped within the cells [13,15,94,95]. Hence, the degradation rate of Lp(a) (or LDL) in aortic intima–inner media can be calculated from the difference in the accumulation of ^{131}I and ^{125}I in that tissue after correction for differences in plasma radioactivity concentrations of the two isotopes [13,15,94,95].

The degradation rate of Lp(a) was significantly smaller (average, 39%) than that of LDL in nonlesioned aortic intima–inner media of rabbits (Fig. 5) [84]. It is quite possible that this finding reflects less efficient degradation of Lp(a) than of LDL via the LDL receptor for the following reasons: cell culture studies strongly indicate that Lp(a) is degraded less efficiently than LDL via LDL receptors [96] and LDL receptor–mediated degradation is a major pathway of LDL degradation in nonlesioned aortic intima of rabbits [13]. Also, the in vivo degradation rate of Lp(a) was lower than that of LDL in tissues that degrade LDL predominantly via LDL receptors (adrenal and intestine) [95].

It is unknown whether the divergent metabolism of Lp(a) and LDL in the nonlesioned intima–inner media affects their relative potential to initiate atherogenesis. Theoretically, slow degradation of Lp(a) could lead to the accumulation of undegraded Lp(a) particles in the intima. However, this idea was not supported by the finding of a slightly lower accumulation of labeled Lp(a) than of labeled LDL in nonlesioned aortic intima–inner media 23 h after intravenous coinjection of labeled Lp(a) and LDL [62]. Thus, it is likely that the major fraction of newly entered Lp(a) that escapes degradation in nonlesioned aortas of rabbits rapidly reenters the plasma compartment instead of accumulating in the arterial wall.

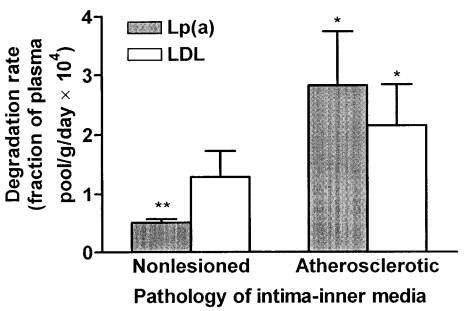

Fig. 5. Degradation rate of Lp(a) and LDL in the intima–inner media of the proximal segment of the aortic arch of nonlesioned and atherosclerotic aorta of rabbits. Human Lp(a) or LDL was doubly labeled with ^{125}I and ^{131}I-tyramine cellobiose and injected intravenously into cholesterol-fed rabbits 24 h before removal of the aorta. Degradation rates in nonlesioned and atherosclerotic intima–inner media were determined in rabbits that had been cholesterol-fed for 1 week and ~6 months, respectively. Degradation rates of Lp(a) and LDL were calculated from arterial wall and plasma radioactivity concentrations as described; Lp(a), $n=7$ and $n=5$ in the nonlesioned and atherosclerotic group, respectively; LDL, $n=11$ and $n=10$ in the nonlesioned and atherosclerotic group, respectively.*$P=0.05$ vs. LDL in nonlesioned intima–inner media and **$P=0.05$ versus atherosclerotic intima–inner media. Values are means ± S.E.M. The depicted data are adapted from [84] with permission.

4.2. Degradation of Lp(a) by foam cells in atherosclerotic lesions

Three h after an intravenous injection of human Lp(a), abundant human apo(a) immunoreactivity can be detected in macrophage-derived foam cells of atherosclerotic lesions in cholesterol-fed rabbits [60], indicating that foam cells internalize and degrade newly entered Lp(a) in the arterial wall in vivo. Additional insight into this process was obtained from the determination of degradation rates of Lp(a) and LDL in atherosclerotic aortas of cholesterol-fed rabbits [84].

The degradation rate of Lp(a) in atherosclerotic intima–inner media of the proximal segment of aorta was more than fivefold higher than in nonlesioned intima–inner media in the same aortic segment (Fig. 5) [84]. The difference in degradation rates of Lp(a) between nonlesioned and atherosclerotic aortas was confined to the intima–inner media (similar Lp(a) degradation rates were observed in the outer media) and was tightly correlated with the extent of cholesterol accumulation in the atherosclerotic intima (and presumably with foam cell abundance) [84]. Thus, in conjunction with the previously mentioned immunohistochemical studies [60], these results are compatible with the idea of rapid Lp(a) degradation in atherosclerotic lesions of rabbits by macrophage-derived foam cells.

The degradation rate of Lp(a) in atherosclerotic aortic intima–inner media was as high as that of LDL (Fig. 5). Because degradation of LDL by macrophages can lead to foam cell formation [76] and because Lp(a) contains cholesterol and cholesterol esters, Lp(a) particles, like LDL particles, may contribute to foam cell formation in atherosclerotic lesions. It is also conceivable, however, that degradation of Lp(a) by macrophages may also serve as a protective mechanism to remove Lp(a) particles from the extracellular milieu in the intima.

The studies of arterial wall Lp(a) degradation rates may be looked upon together with studies of intimal clearance of labeled Lp(a) [63] to gain some insight into the importance of degradation in the removal of newly entered Lp(a) from the arterial intima–inner media. The fraction of newly entered Lp(a) removed from the aortic intima–inner media by degradation was assessed by converting the degradation rate from fraction of plasma pool per g per day to nl per cm^2 per day. The average Lp(a) degradation rate in the intima–inner media (fraction of plasma pool/g per day) [84] was multiplied by the total plasma volume of the rabbits (40×10^3 μl/kg × 3.5 kg) and by the weight of the intima–inner media specimens (g/cm^2 surface area). Because the degradation rate and intimal clearance of Lp(a) both depend on the severity of atherosclerosis [60,84], this calculation was only performed for the proximal segment of the aortic arch, where the mean

cholesterol content was similar in the atherosclerotic rabbits used to study degradation rates and in those used to study intimal clearance. From these calculations, the fraction of newly entered Lp(a) removed by degradation was 18 and 23% in the nonlesioned and atherosclerotic intima–inner media, respectively. This suggests that cellular metabolism of Lp(a) is an important mechanism for removal of Lp(a) from the intima, although the larger fraction (~ 80%) of newly entered Lp(a) probably leaves the arterial wall as intact Lp(a) particles. Whether this conclusion is valid in humans is unknown. Rabbits do not have Lp(a) and their metabolism of Lp(a) may be different from that in humans. However, the degradation rate of Lp(a) in the rabbit aorta was not affected by increasing the plasma Lp(a) concentration by an intravenous injection of human Lp(a) [84]. At this stage, no study has determined Lp(a) or LDL degradation rates in human arterial tissue.

4.3. Oxidation of plasma LDL accelerates its degradation in nonlesioned aortic intima–inner media

To investigate the effect of oxidation of LDL on its metabolism in nonlesioned rabbit aorta in vivo, Juul et al. compared the degradation rates of ox-LDL and normal LDL using the TC method [66]. The degradation rate of ox-LDL was markedly greater than that of normal LDL, both in the intima–inner media (Fig. 3) and in the outer media of the aorta. There are no foam cells in nonlesioned rabbit aorta [11,12]. Thus, smooth muscle cells and possibly endothelial cells seemingly are capable of taking up and degrading ox-LDL in nonlesioned arterial intima and may play an important role in the metabolism of ox-LDL in nonlesioned arterial intima–inner media. This notion contrasts with the situation in atherosclerotic lesions, where acetylated LDL is predominantly degraded by foam cells [14].

5. Conclusions and perspectives

The studies reviewed here support the idea that the same factors govern the transport rates of Lp(a) and LDL from plasma into the arterial intima and that the plasma Lp(a) concentration is a major determinant of the mass flux of Lp(a) into the arterial wall [60,61,63]. Additionally, the pathological state of the arterial wall may also have pronounced effects on Lp(a) accumulation in the intima–inner media: development of atherosclerosis in the proximal segment of the aorta in cholesterol-fed rabbits was accompanied by an 800% increase in arterial wall intimal clearance and a 75% decrease in fractional loss of Lp(a) (Fig. 6) [63].

The mechanisms that lead to increased intimal clearance and decreased fractional loss of Lp(a) in

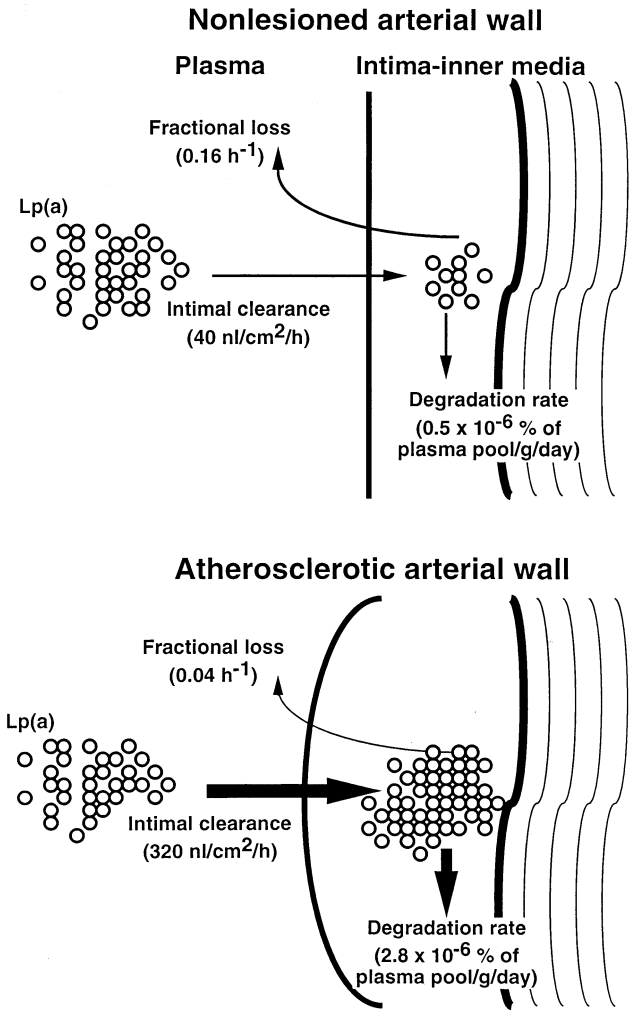

Fig. 6. Model of Lp(a) interaction with nonlesioned and atherosclerotic intima–inner media. Atherosclerosis is accompanied by marked changes in the metabolism of Lp(a) in the arterial wall. An increased intimal clearance (and thus influx) of Lp(a) as well as a decreased fractional loss (and thus increased mean residence time in the arterial wall) both contribute to increased accumulation of Lp(a) in atherosclerotic lesions. Furthermore, Lp(a) degradation rates are increased in atherosclerotic lesions, presumably reflecting uptake and degradation of Lp(a) by macrophage-derived foam cells. Numbers in parentheses are rate constants for interaction of human Lp(a) with nonlesioned (top panel) or atherosclerotic (bottom panel) intima–inner media in the proximal segment of the aorta in rabbits.

atherosclerotic lesions of rabbits are unknown. Ultrastructural analyses indicate that atherosclerotic lesions are covered by morphologically intact endothelium [1,9,97]. A challenge in the future will be to dissect the molecular and structural changes of the endothelium that cause it to become more permeable to plasma lipoproteins at sites of atherosclerosis. Similarly, it will be of importance to identify the components of atherosclerotic lesions that cause decreased fractional loss of Lp(a). Mice with induced mutations in the fibrinogen gene [98] and possibly in the future mice lacking specific glycosaminoglycans will be useful for

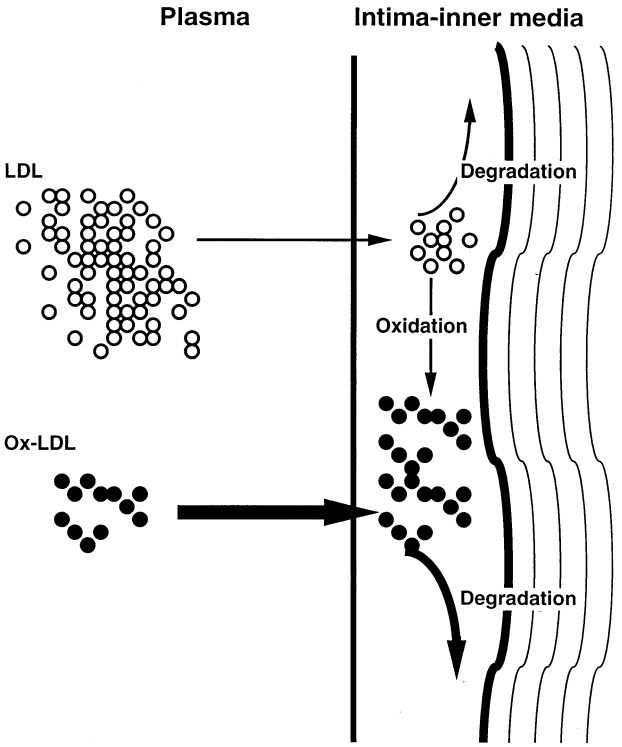

Fig. 7. Model of ox-LDL accumulation in the arterial intima–inner media. Ox-LDL in the arterial wall can be derived either from normal LDL that becomes oxidized after its entry into the arterial intima–inner media or from ox-LDL in plasma. Ox-LDL in plasma enters, accumulates and is degraded in aortic intima–inner media of rabbits faster than normal LDL.

testing the importance of these molecules in the retention of Lp(a) in the arterial intima in vivo. Further insight into the interactions of Lp(a) and arterial wall components may suggest novel targets for therapies to prevent atherosclerosis, particularly because the mechanisms that cause accelerated influx and decreased efflux of Lp(a) conceivably could also affect the metabolism of LDL and other atherogenic lipoproteins in the arterial wall.

Studies in rabbits suggest that Lp(a) can be taken up and degraded by macrophages in the arterial intima in vivo [60,84]. An important task will be to determine the mechanisms for this finding. Studies of the Lp(a) degradation by macrophages lacking the class A scavenger receptors [99] may be useful for testing the importance of these receptors in Lp(a) metabolism. Similarly, efforts to clone a specific Lp(a) receptor expressed by foam cells [100–102] may provide the opportunity to design studies that address the role of that receptor in Lp(a)—macrophage interactions and ultimately in atherosclerosis.

The fractional loss of Lp(a) was 27% of that of LDL in balloon-injured rabbit aorta and the accumulation of labeled Lp(a) in balloon-injured intima–inner media exceeded that of LDL after a 23-h exposure to the labeled lipoproteins [62]. If these results can be extrapolated to humans, then retention of Lp(a) may cause specific accumulation of Lp(a) rather than of LDL in arteries after angioplasty. Lp(a) has been reported to accelerate smooth muscle cell proliferation [38] and downregulate glucocorticoid receptors in cultured human and rat smooth muscle cells [103]; both effects might contribute to restenosis after angioplasty. Of note, several (but not all) studies indicate that a high plasma Lp(a) concentration is a risk factor for restenosis after angioplasty of atherosclerotic coronary arteries [104–110].

Future studies are needed to identify the biochemical features of Lp(a) that might cause it to accumulate at sites of arterial injury. Use of adenovirus-mediated gene transfer to express mutated forms of apo(a) in mice [111] provides a unique approach for obtaining insight into the molecular features of apo(a) involved in Lp(a) binding to arterial wall components. Interestingly, recent studies have suggested that the lysine-binding properties of apo(a) may be an essential factor for accumulation of Lp(a) in the arterial intima and accelerated atherogenesis in transgenic mice [111,112]. Another potentially important question, which cannot be answered from the currently available studies, is whether different apo(a) isoforms are metabolized differently in the arterial wall.

Recent studies in rabbits support the idea that mildly oxidized LDL can circulate in plasma for prolonged periods and that ox-LDL particles enter, accumulate and are degraded in the arterial intima in preference to normal LDL [66]. The idea that plasma-derived ox-LDL accumulates in the arterial wall complements the previous proposition that ox-LDL in the arterial intima is derived from normal LDL, oxidized after its entry into the arterial intima (Fig. 7). Because assays to quantitate ox-LDL in plasma are becoming available [54], it will be possible to determine whether a high plasma level of ox-LDL in plasma is associated with an increased risk of atherosclerosis.

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