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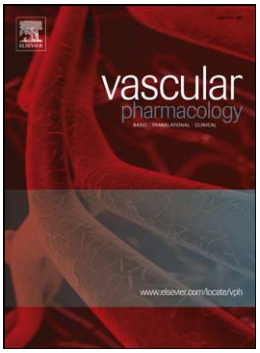

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PII: S1537-1891(15)30101-4
DOI: doi: 10.1016/j.vph.2016.05.013
Reference: VPH 6325

To appear in: *Vascular Pharmacology*

Received date: 11 December 2015
Revised date: 26 April 2016
Accepted date: 28 May 2016

Physiology and pathophysiology of oxLDL uptake by vascular wall cells in atherosclerosis

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KEY WORDS: oxidized low-density lipoproteins, macrophages, atherosclerosis.

Abstract

Atherosclerosis is a progressive disease in which endothelial cell dysfunction, macrophage foam cell formation, and smooth muscle cell migration and proliferation, lead to the loss of vascular homeostasis. Oxidized low-density lipoprotein (OxLDL) may play a pre-eminent function in atherosclerotic lesion formation, even if their role is still debated. Several types of scavenger receptors (SRs) such as SR-AI/II, SRBI, CD36, lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), toll-like receptors (TLRs) and others can promote the internalization of oxLDL. They are expressed on the surface of vascular wall cells (endothelial cells, macrophages and smooth muscle cells) and they mediate the cellular effects of OxLDL.

The key influence of both OxLDL and SRs on the atherogenic process has been established in atherosclerosis-prone animals, in which antioxidant treatment and/or silencing of SRs has been shown to reduce atherogenesis.

Despite some discrepancies, the indication from cohort studies that there is an association between oxLDL and cardiovascular (CV) events seems to point towards a role for oxLDL in atherosclerotic plaque progress and disruption.

Finally, randomized clinical trials using antioxidants have demonstrated benefits only in high-risk patients, suggesting that additional proofs are still needed to better define the involvement of each type of modified LDL in the development of atherosclerosis.

Introduction

Atherosclerosis is a chronic and progressive disease, its development is due to the impairment of several molecular and cellular activities leading to the gradual loss of structural and functional vascular homeostasis. In particular, endothelial cell dysfunction has been associated with the development of atherosclerotic lesions and, in the early stage of atherogenesis, it is characterized by the increasing expression of vascular adhesion molecules and/or chemoattractants, which promotes adhesion of circulating monocytes to the endothelium [1, 2]. Following adhesion, the monocytes migrate into the intimal layer of the vascular wall and differentiate into macrophages, a key initiating factor in early atherogenesis characterized by the development of fatty streaks [3]. The intimal macrophages present scavenger receptors able to recognize and internalize excess lipid derived from modified low-density lipoproteins (mLDL) and/or oxidized low-density lipoproteins (oxLDL), which arise from oxidation of plasma LDL cholesterols, favouring the intracellular accumulation of cholesterol esters and generation of foam cells [4].

Recently, it has been proven that several types of macrophage SRs such as class A SRs (SR-AI, SR-AII, SR-AIII), class B SRs (SR-BI, SR-BII, CD36), class E lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and others can promote the internalization of oxLDL. This, as mentioned above, can lead to excessive sub-endothelial localization of foam cells, development of fatty streaks, and consequently plaque formation [5].

In this review, since the hypothesis that oxidative stress plays a central role in atherosclerosis initiation and progression is still debated [6], we briefly summarize the evidence for the hypothesis whereby foam cell formation is due mainly to dysregulated uptake of oxLDL by macrophages, and go on to focus on the cellular and molecular pathways potentially involved in this phenomenon.

Interestingly, in this issue of Vascular Pharmacology, using an *in vitro* (murine macrophages) and *in vivo* murine model of atherosclerosis (ApoE-/- mice on a high-cholesterol high-fat diet), Chen and co-authors provide experimental evidence to suggest a novel mechanism of LOX-1 regulation

by adenosine monophosphate-activated protein kinase (AMPK) inhibiting macrophage oxLDL uptake and atherosclerosis [7]. This data further support several studies, which have provided proof of concept for the idea that AMPK is protective in cardiovascular system and that AMPK signalling dysfunction is involved in several mechanisms implicated in the genesis and development of various cardiovascular diseases, including atherosclerosis [8]. Notably, in the study by Chen et al [7] the authors show that *in vivo,* pharmacological AMPK activation reduces the atherosclerosis lesion size and the expression of LOX-1 in the aortas of apolipoprotein E-deficient mice, suggesting a new insight into the potential protective role of AMPK in vascular LOX-1 regulation *in vitro* and *in vivo* [7]. This provides new impetus for further investigations designed to characterize the compounds involved, which would open new perspectives in regulating oxLDL uptake in macrophages through the AMPK/PP2A/NF-κB/LOX-1 pathway.

***In vitro* evidence linking oxidized LDL to Atherosclerosis.**

OxLDL can actively participate in the atherosclerotic process by various mechanisms, including the induction of endothelial cell (EC) activation and dysfunction, macrophage foam cell formation, and vascular smooth muscle cell (VSMC) migration and proliferation [9].

The effect of oxLDL on ECs is mediated by two scavenger receptors known as cluster of differentiation 36 (CD36, also known as fatty acid translocase) and LOX1 [10, 11]. CD36 seems to play a limited role in the pathogenesis of macroangiopathy, while it is more involved in microangiopathy and unstable atherosclerotic disease, especially in the presence of co-morbidities such as type 2 diabetes and insulin resistance.

Indeed, CD36 seems not be expressed in macrovascular ECs, wherease it represents the main oxLDL-binding receptor in microvascular ECs [12]. In these cells, high insulin levels increase CD36 protein expression [13, 14], mimicking hyperinsulinemia and the simultaneous exposure to fatty acids, such as occurring in conditions of insulin resistance and diabetes.

On the other hand, LOX-1 is expressed in several pro-inflammatory conditions and seems to play a crucial role in the early stage of plaque formation [5, 15].

Recently, oxLDL was found to upregulate LOX-1 expression in the ECs, suggesting that oxLDL modulates its own receptor through interaction with LOX-1 [5]. OxLDL formed and retained in the sub-endothelial space activates ECs through induction of cell surface adhesion molecules that in turn induce the rolling and adhesion of blood monocytes and T cells. Also, up-regulation of endothelial adhesion molecules, such as intercellular- and vascular cell adhesion molecule-1 (ICAM-1 and VCAM-1), can be induced by oxLDL in an LOX-1-dependent manner and this is mediated by the nuclear factor κB (NF-κB) [16, 17]. In addition, OxLDL is itself a chemoattractant for monocytes [18] and stimulates endothelial release of monocyte chemotactic protein-1 (MCP-1) [19].

More recently, it has been demonstrated that this effect was inhibited by LOX-1 knockdown involving the mitogen-activated protein kinase (MAPK) pathway [20]. oxLDL was also found to inhibit the expression of endothelial nitric oxide synthase (eNOS), and to reduce nitric oxide (NO) bioavailability by increasing reactive oxygen species (ROS) production [21, 22]. The inhibitory effects on endothelial NO production have been associated with LOX-1 function [17, 23].

Another important effect of oxLDL is that, by enhanced sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) oxidation, it induces aberrant endoplasmic reticulum (ER) stress in bovine aortic ECs (BAECs) [24]. This effect is inhibited by AMP-activated protein kinase (AMPK) activation, suggesting that AMPK is an important regulator in the atherosclerotic process [24]. Furthermore, it has recently been proved that oxLDL can induce endothelial cell death through the activation of NF-κB and activator protein-1 (AP-1) pathways [25], worsening endothelial dysfunction and promoting the progression of atherosclerotic plaque.

By releasing pro-inflammatory signals and through the abnormal hemodynamic force and/or accumulation of oxLDL in the arterial wall, the inflamed and atheroprone endothelium attracts monocytes that infiltrate the intima [26]. In the sub-endothelial layer, monocytes differentiate to

macrophages that in turn polarize to pro-inflammatory/anti-inflammatory phenotypes depending on local stimuli and transforming into foam cells [27].

Increased uptake of oxLDL and/or reduced cholesterol efflux leads to the deposition of esterified cholesterol in the cytoplasm of macrophages and generation of foam cells [28]. Macrophages internalize oxLDL by several SRs such as SR-AI/II, SRBI, CD36, LOX-1 and toll-like receptors (TLRs) [5, 29]. Once activated, macrophages induce the release of pro-inflammatory cytokines (interleukin 1-β, tumor necrosis factor), reactive oxygen species (ROS) and metalloproteases, which are associated with progression of inflammation [30].

Internalized oxLDL provides oxidized lipids as ligands for the PPAR-γ pathway, upregulating CD36 expression, and in turn facilitating the internalization of more oxLDL [31, 32]. These molecular events activate the macrophages, causing secretion of both cytokines, which recruit immune cells to the intima, and the enzymes myeloperoxidase and 12/15-lipoxigenase, which are thought to participate in the oxidization of new LDL, increasing the local pool of oxLDL [33, 34]. Also, the internalization of oxLDL by CD36 seems to induce inhibition of macrophage migration, favoring cell spreading and the activation of focal adhesion kinase, in a process mediated by src-kinases and oxidative stress [35]. Besides, oxLDL-CD36 interaction causes a loss of cell polarization in macrophages, an essential process toward cell migration [36]. Thus, the evidence suggests that oxLDL participates not only in monocyte differentiation and macrophage activation, but also macrophage retention.

As previous mentioned, LOX-1 is one of the SRs expressed in macrophages and when it occurs, by the influence of pro-inflammatory cytokines, oxLDL or other stimuli, the oxLDL uptake increases significantly favoring foam cell formation [5, 37]. Moreover, proinflammatory cytokines upregulate LOX-1 and downregulate other SRs (SR-AI/II and CD36), suggesting that, in inflamed microenvironments, where these cytokines are relatively abundant (such as in atherosclerotic lesions), LOX-1 might play a significant role in macrophage OxLDL uptake [5].

Interestingly, in this issue of Vascular Pharmacology, Chen and co-authors have shown, in murine macrophage J774A.1 cells, that AMPK plays a crucial role in reducing cholesterol uptake. Briefly, pharmacological activation of AMPK has been shown to promote protein phosphatase 2A (PP2A) activity and consequently to decrease NF-κB/LOX-1 phosphorylation/expression [7]. This finding suggests a novel mechanism of LOX-1 regulation by AMPK, which attenuates macrophage oxLDL uptake and atherosclerosis. Moreover, AMPK together with sterol regulatory element binding transcription factor 1 (SREBP1), was recently shown to be involved in the decrease of foam cell formation in mouse macrophage Raw264.7 cells treated with liraglutide [38]. Additionally, AMPK suppresses oxLDL induced macrophage proliferation, which is a key event underlying the development of atherosclerosis [39]. These findings together with others on endothelium [24], prove that AMPK may prevent the uptake of oxLDL in both macrophages and ECs, suggesting it has a crucial role in modulating the atherosclerotic process.

Aside from this evidence, several studies, which has already been reviewed by Chistiakov and co-authors, have shown that various different nutrients and dietary components are able to regulate the oxLDL uptake in macrophages through modulation of the major scavenger-receptors (CD36, SR-A1 and LOX-1) [40]. More recently, the flavonoid quercetin was shown to prevent lipid accumulation in mouse macrophage Raw264.7 cells through modulation of ER-stress pathways [41].

Along with the dysregulated uptake of oxLDL in macrophages, another key point in the formation of foam cells is the regulation of cholesterol efflux. Various transporters such as Acyl coenzyme A-cholesterol acyltransferase-1 (ACAT1) and neutral cholesteryl ester hydrolase (nCEH) [42], ATP-binding cassette (ABC) transporters ABCA1 and ABCG1, and SR-BI contribute to the reverse transport of cholesterol from macrophages [40, 43, 44]. Briefly, ABCA1 promotes cholesterol efflux to lipid-poor APOA1, which is the building block of HDL, whereas ABCG1 promotes efflux to mature HDL particles. The genes encoding ABCA1 and ABCG1 are transcriptionally upregulated in response to elevated cellular cholesterol levels by liver X receptors (LXRs), which

are ligand-activated nuclear receptors that act as sterol sensors [45, 46]. Thus, synthetic LXR agonists have been actively investigated for the treatment of atherosclerosis. More recently, in human THP1 macrophages, the activation of G protein-coupled receptor GPR55 was shown to increase CD36- and SRB-I- mediated lipid accumulation and to block ABCA1 and ABCG1 cholesterol efflux, suggesting it plays a deleterious role in oxLDL-induced foam cell formation.

Like macrophages, VSMCs also express SRs, among which LOX-1, and could convert to foam cells on exposure to lipoproteins [47-50]. In VSMCs, oxLDL promotes a phenotypic switch toward the proinflammatory phenotype associated with their de-differentiation, proliferation and migration [51]. The proliferation of VSMCs can be stimulated by oxLDL, since these particles enhance platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) expression and secretion [52] by ECs and macrophages. On the other hand, oxLDL also induces the secretion of a variety of other growth factors and their receptors: insulin-like growth factor-1(IGF-1) and epidermal growth factor (EGF), all with mitogenic effects inducing SMC proliferation [53]. Interestingly, high concentrations of oxLDL cause LOX-1-mediated apoptosis of VSMCs by increasing expression of Bax and suppressing B lymphoma-2 (Bcl-2), an anti-apoptotic factor, and by contributing to plaque instability [54, 55]. Notably, deletion of LOX-1 reduces atherogenesis and is associated with reduction in proinflammatory and prooxidant signals [55-57]. Incubation of cultured bovine VSMC with lysophosphatidylcholine (LPC), an atherogenic component of oxLDL, resulted in upregulated LOX-1 expression and increased oxLDL uptake [58]. Furthermore, oxLDL was shown to prevent PDGF-induced differentiation of SM progenitor cells to SM-like cells and increase lipid accumulation in cytoplasm [59]. In addition, like in endothelial cells and macrophages, an anti-atherosclerotic role of AMPK has been also reported in VSMCs, in which AMPK activation by resveratrol can block the oxLDL stimulated PI3K/Akt/mTOR/p70S6K pathway with consequent inhibition of DNA synthesis and proliferation of smooth muscle cells [60].

Taken together, these findings suggest a likely role of oxLDL in mediating transformation of VSMCs into foam cells. Hence, oxLDL contributes to proatherogenic activation of VSMCs and this is associated with increased lipid intake, apoptosis, proliferation and migration.

In conclusion, oxLDL and its receptors, in particular LOX-1, appear to be star contributors to the formation of atheroma and the rupture of atherosclerotic plaques by acting upon cultured macrophages and ECs as well as VSMCs.

***In vivo* models supporting the role of oxidized LDL in Atherosclerosis**

Several findings obtained in *in vivo* porcine models suggest that these models can have high relevance for the human situation of raised cardiovascular risk factors [61] such as elevated oxLDL, which is associated with atherosclerosis and high CV risk. Recently, Holvoet and colleagues [62] identified 18 genes in coronary plaque macrophages of hypercholesterolemic pigs that correlated with plaque oxLDL. In the same experimental porcine model oxLDL-induced expression of ABCA1 in blood monocytes, which precedes coronary atherosclerosis and it is associated with plaque complexity [63]. Additionally, oxidized LDL/LOX-1/NF-B signaling axis has been shown to be involved in the early initiation of a juvenile obesity-induced proatherogenic coronary artery phenotype in Ossabaw pigs [64].

Interestingly, in the last few years several *in vivo* studies have been carried out in other animal models where either modulation of oxidative stress [65] or manipulation of the scavenger receptor was undertaken, in order to better verify the role of oxidized LDL in the pathogenesis of atherosclerosis,.

Several years ago Witztum and Steinberg [66] reviewed the data and studies showing the presence of oxidized LDL in *in vivo* animal models, confirming that inhibition of oxidation by pharmacologic and/or genetic manipulations retards atherogenesis. Most of these studies were carried out with different types of antioxidants such as probucol and in this case established a protection from atherosclerotic lesions, with the exception of murine models [67]. For a long time,

vitamin E was assumed to take effect in animal models by decreasing the oxidation of LDL, a key step in atherosclerosis initiation [68]. However, at the cellular level, vitamin E acts by inhibition of many reactions involved in the progression of atherosclerosis and recent studies revealed that these effects are not the result of vitamin E antioxidant activity, but rather of precise molecular actions by this compound [69].

Recently, Cui and colleagues [70] demonstrated that another potent antioxidant, N-acetylcysteine (NAC), attenuated *in vivo* oxidation of native LDL and ROS formation from ox-LDL that also significantly reduced atherosclerotic plaque formation in hyperlipidemic LDL receptor knockout (LDLR−/−). In addition, in a model of quails (Coturnix coturnix) raised on a high fat diet it was found that Oleanolic acid (OA) could potentially alleviate high fat diet induced atherosclerosis through a mechanism which involves modulation of LOX-1 activity, including inhibiting the expression of NADPH oxidase subunits [71].

Furthermore, a recent study has demonstrated that insulin-like growth factor I (IGF-1) infusion in Apoe (-/-) mice decreased atherosclerotic plaque size, as well as plaque macrophage and lipid content, via downregulation of 12/15-lipoxygenase (12/15-LOX). This mechanism may play a major role in the anti-atherosclerotic effects of IGF-1 [72].

In remarkable further support of the study by Chen et al. [7] published in this issue of Vascular Pharmacology, Dong and co-workers [24] demonstrated that chronic administration of Tempol (a potent antioxidant) combined with AMPK activation is able to suppress oxidized and glycated LDL (HOG-LDL)–induced endoplasmic reticulum (ER) stress by inhibiting NAD(P)H oxidase–derived ROS, SERCA oxidation and atherosclerosis in $ApoE^{-/-}$ and $ApoE^{-/-}$/AMPKα$2^{-/-}$ mice fed on a high-fat diet. In addition, reduction of AMPK has been shown to increase endoplasmic reticulum stress and atherosclerosis in AMPKα2 knockout mice [73]. These latter studies highlight the role of AMPK as physiological regulator able to maintain ER homeostasis and endothelial function, and suggest the involvement of AMPK in the pathophysiological process of atherosclerosis.

Although support for the oxidative theory comes from broad-ranging literature on the treatment of atherosclerosis-prone animals with antioxidants, the results of the various studies have proved to be highly contradictory, due to the variety of animal models used, the different genetic background, and the unexpected effects of gene deletions [74].

Thus, even though in an animal model of increased oxidative stress, obtained through the overexpression of 15-lipoxygenase in the vascular wall, large atherosclerotic lesions were found in LDL receptor-deficient mice [75], decreased atherosclerosis was also reported in cholesterol-fed rabbits and WHHL [76] rabbits whose macrophages overexpressed human 15-lipoxygenase [77]. Moreover, though three different knockout mouse models for 12/15-lipoxygenase showed a decreased severity of atherosclerosis [34, 78-80] in apoE-deficient mice, knocking out the macrophage-specific 12/15-lipoxygenase increased the extent of atherosclerotic lesions [81]. Accumulated evidence showed that OxLDL can induce ROS production and release inflammatory factors via scavenger receptors, such as CD36 and LOX-1 [76, 82]. When either SR-A or CD36 scavenger receptors, accounting for almost 90% of macrophage oxidized LDL uptake were knocked out in atherosclerosis-prone mice models [83], it proved to be efficacious in decreasing the atherosclerotic burden [84, 85]. However, even though double knockout of SR-A and CD36 in ApoE2/2 mice led to a reduction in peritoneal macrophage lipid accumulation, the reduction of atherosclerosis was not confirmed [76]. Lately, Makinen and co-workers [86] have shown that silencing either SR-A or CD36 reduces atherosclerosis in hyperlipidemic mice and reveals a reciprocal upregulation of these receptors. Note that these authors studied the role of SR-A and CD36 in foam cell formation and atherogenesis by RNA interference (RNAi)-mediated silencing, which is a clinically feasible method of down-regulating the expression of these receptors.

Manning-Tobin and colleagues demonstrated that, although targeted deletion of SR-A and CD36 does not abrogate macrophage foam cell formation or substantially reduce atherosclerotic lesion area in Apoe-/- mice, loss of these pathways does reduce progression to more advanced necrotic

lesions. These data suggest that targeted inhibition of these pathways *in vivo* may reduce inflammation and promote plaque stability [87].

Besides scavenger receptors, another receptor of interest, which OxLDL has been shown to transactivate, is epidermal growth factor receptor [88-90]. Interestingly, Meprins were discovered to be membrane-bound metalloproteinases [91] which are involved in inflammation [92-95]. Recently, Gao and colleagues [96] demonstrated that Meprinα promotes OxLDL-induced plaque formation in high-fat diet apolipoprotein E-deficient (apoE– /–) mice and in cultured J774a.1 macrophage ROS release by transactivation of the EGFR, followed by activation of the PI3K/Rac1/p38 pathway.

Finally, since air pollution and nicotine exposure have been shown to potentiate plaque progression in humans and animals [97-99], this review also summarizes the data showing that in atherosclerosis-prone ApoE−/− or LDLR−/− mice nicotine boosts the proatherogenic effects of oxLDL by stimulating and upregulating macrophage CD36 signaling [100], while CD36-dependent 7-Ketocholesterol accumulation in macrophages mediates progression of atherosclerosis in response to chronic air pollution exposure [101].

Oxidized LDL and atherosclerosis development: lessons from studies in humans

In humans atherosclerosis is characterized by a complex aetiology with multiple factors contributing to the pathogenesis of the disease, and both genetic predisposition and environmental factors involved in atherosclerotic lesion initiation and progression [102].

The Framingham study identified a host of risk factors for atherosclerosis including hypertension, hypercholesterolemia, diabetes mellitus, obesity, smoking, family history, and inactive lifestyles. These risk factors are thought to contribute to the onset and progression of atherosclerosis by disrupting a number of lipid regulatory and inflammatory mechanisms within the arterial wall [103].

The cholesterol in atherosclerotic lesions originates in the circulation; LDL-cholesterol is pro-atherogenic, but LDL has to be modified to promote atherosclerosis [104]. Over the last few decades it has become evident that oxidatively modified LDL plays a major role in the development

and progression of atherosclerosis and its complications [53] but recently multiple evidence has tended to suggest that LDL may be modified by other mechanisms in addition to oxidative changes [105].

Many population studies have reported a positive association between increased oxLDL levels and CV risk factors, including hypercholesterolemia, hypertriglyceridemia, metabolic syndrome, obesity, diabetes, insulin resistance, hypertension, and severe kidney disease [35, 106-108], suggesting that oxidized LDL is biologically relevant for the development of metabolic disorders and then Coronary Artery Disease (CAD).

Increased oxLDL levels have also been reported in plasma from patients with angiographically documented atherosclerosis and have been associated with the severity of CAD [108].

However, although the involvement of oxLDL in the development of atherosclerosis is widely accepted, its value as an independent biomarker of CVR is only moderate.

Human studies on the association of oxidized LDLs with atherosclerosis or cardiovascular events have been highly conflicting [109]. Some cross-sectional studies have suggested a direct association between oxidized LDLs or oxLDL antibodies and atherosclerosis in various vascular beds [110-112], whereas others have found no association with the coronary atherosclerotic burden in coronary artery disease patients.

Most cohort studies report an association between oxidized LDLs and cardiovascular events or mortality, in particular those including either a very high-risk population, such as patients with end-stage renal disease and diabetes, or coronary artery disease patients [4].

Unfortunately, despite being an appealing hypothesis, the oxidation theory of atherosclerosis is not conclusively corroborated by consistent results, probably due to different possible reasons: a) patient selection bias, e.i. differences in risk profiles of patients included in human trials and degree of adjustment for other cardiovascular risk factors; b) low statistical studies power and c) the lack of reliable uniform methods to quantify oxLDL *in vivo*; circulating oxLDL can indeed be detected either by quantifying the immunogenic response against them (oxLDL antibodies) or directly

measuring oxLDL epitopes, both methods presenting pros and cons [113]; thus complete standardization of the LDL quantification technique is necessary if we are to compare results from different clinical trials.

Moreover, the oxidative theory of atherosclerosis would be conclusively proven by the beneficial effects of oxidative stress decrease on cardiovascular events, except that most controlled randomized trials involving the use of antioxidants have provided negative results [4]. Interestingly, beneficial effects from antioxidant supplementation have been observed in patients with either known cardiovascular disease or with a very high-risk profile, thus indicating that antioxidant supplement administration to low-risk profile patients does not provide cardiovascular protection [114].

The lack of positive results in large clinical trials using different antioxidant molecules has also contributed to fostering the hypothesis that LDL retained in the arterial wall could be modified not only by lipid peroxidation but also by ubiquitous hydrolytic enzymes [66, 115].

The current general perception is that, while it is still accepted that lipid peroxidation plays a significant role in atherogenesis, other mechanisms of LDL modification, such as enzymatic modification through lipases or proteases, may play a significant role in generating modified LDL within the arterial wall [116, 117]. This is consistent with the recent view that atherosclerosis is a chronic inflammatory disease in the artery walls, which stems from the interaction between arterial cells, lipoproteins and inflammatory cells, leading to the development of complex lesions or plaques [8]. Hence, as proposed in this issue of Vascular Pharmacology by Chen et al. [7], a deeper understanding of the mechanisms leading to macrophagic uptake of modified LDL could turn out to be very helpful when it comes to enacting new therapeutic strategies against atherosclerosis. In a mouse model of atherosclerosis, the same authors showed a potential positive action by AMPK activators in reducing atherosclerotic lesion progression over and above their lipid-lowering effects, [7], suggesting that AMPK protects the vascular wall from atherosclerosis.

The occurrence of modified LDL forms in plasma may also reflect the silent presence of active atherosclerotic lesions, because LDL is oxidized and/or modified in injured areas of the arterial wall but is not found in healthy areas [118]. In accordance with that view, the mRNA of scavenger receptor LOX-1 has been found in human atherosclerotic plaques while negligible amounts are detectable in unaffected aortas [15]. Circulating oxLDL transiently and abundantly increase during the acute phase of cardiovascular events such as acute myocardial infarction or stroke, and also after percutaneous transluminal angiography [112, 119]; this supports the concept that circulating oxLD/modified LDL come from the injured arterial wall and suggest that quantifying them in the blood may be a very helpful tool in increasing our understanding of the vulnerability of atheromatous lesions as well as for secondary prevention of cardiovascular events in patients with atherosclerosis.

Modified LDL may thus be considered a potential indicator of unstable and/or ruptured atherosclerotic plaques releasing part of their contents into circulation. However, additional studies conducted by various different research groups, with a standardized detection method, on patients with different diseases or conditions associated with early atherosclerosis are needed to better define the implication of each type of modified LDL in the development of atherosclerosis.

Conclusions

In conclusion, on molecular grounds, there is considerable evidence to support the oxidative hypothesis of atherosclerosis. Yet when it comes to translating the experimental evidence to humans and trying to demonstrate the association of oxidative stress with cardiovascular events the result has been contrasting findings, particularly evident with administration of antioxidant therapy. However, when higher risk or cardiovascular patients have been selected, the outcome has proved much more rewarding and numerous positive studies may be cited. It therefore seems that although this theory still needs further evidence before we can say it has been definitively clarified, the data available so far do tend to strengthen the pivotal role of oxidative stress in atherosclerosis**.**

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Graphical abstract