Invited critical review

Mechanisms of LDL oxidation

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Abstract

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Backgrounds: Many lines of evidence suggest that oxidized low-density lipoprotein (LDL) is implicated in the pathogenesis of atherosclerotic vascular diseases. This review summarizes a diversity of mechanisms proposed for LDL oxidation serving for the so-called “LDL oxidation hypothesis of atherogenesis”.

Methods and results: We investigated the literature and our research results related to mechanisms of LDL oxidation and its atherogenesis. LDL oxidation is catalyzed by transition metal ions and several free radicals, and LDL is also oxidized by some oxidizing enzymes. In this way, LDL can be converted to a form that is recognized specifically by and with high affinity to macrophage scavenger receptors, leading to foam cell formation, the defining characteristic of fatty streak lesions.

Conclusions: Several pathways are involved in the promotion of LDL oxidation in vitro and in vivo, but it would appear that the physiologically relevant mechanisms of LDL oxidation are still imperfectly understood. The underlying mechanisms of LDL oxidation must be further explored to reveal appropriate ways for the diagnosis and treatment of atherosclerosis and its relevant diseases.

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1. Introduction

Many lines of evidence suggest that oxidation of low-density lipoprotein (LDL) plays an important role in the pathogenesis of atherosclerosis particularly in animal models have been accumulated, and oxidized LDL in circulation is a well-known risk marker of human cardiovascular diseases, principal cause of which is atherosclerosis [1–7]. The earliest and most common lesion of atherosclerosis is the fatty streak, which consists of the subendothelial lipid-laden foam cells, predominantly macrophages. Many studies have reported that statin therapy decreases LDL-cholesterol and thereby prevents cardiovascular disease, and the increased LDL-cholesterol is established as a major risk factor for cardiovascular disease [8–10]. However, LDL-
promoted development of lipid-laden foam cells remains to be still defined. Cholesterol uptake by way of LDL receptor cannot accumulate cholesterol in macrophages because the LDL receptor is down-regulated by the increased intracellular cholesterol through the mechanism of sterol regulatory element binding protein (SREBP). In contrast, certain modified forms of LDL, such as acetylated LDL and oxidized LDL, are avidly taken up by macrophages through scavenger receptors, leading to the substantial cholesterol accumulation and the foam cell formation because the scavenger receptor is rather up-regulated by oxidized LDL [10–15]. The so-called LDL oxidation hypothesis of atherosclerosis predicts that LDL oxidation is an early event in atherosclerosis and that oxidized LDL contributes to atherogenesis. This hypothesis is supported by several evidences that oxidized LDL can develop foam cell formation in vitro; oxidized LDL is present in vivo; oxidized LDL has a number of proatherogenic potentials such as stimulations of endothelial cells and monocytes toward the increased inflammatory cytokines, chemokines and adhesion molecules, stimulations of monocytes/macrophages toward the increased tissue factor, matrix metalloproteinase, and scavenger receptors, eventually leading to foam cell formation of macrophages and the progression of atherosclerotic lesions [1,2,7].

It is believed that LDL oxidation does not take place in the circulation, and must occur in the arterial wall because serum lipoprotein lipids are well protected from oxidation by the robust antioxidant defenses and LDL itself contains most of alpha-tocopherol, an antioxidant vitamin, as a major transport vehicle [7,16,17]. LDL may be exposed to cell-derived oxidants in the subendothelial space of artery (Fig. 1). LDL can be oxidized non-enzymatically by transition metal ions, hemin, and many other catalysts. On the other hand, there are many postulated mechanisms by which LDL could become oxidized via several enzymes within the artery wall. LDL particles undergo the oxidative modification by incubating with macrophages, endothelial cells and smooth muscle cells [7,18–22]. This review summarizes and discusses shortly oxidized components of LDL and mainly the mechanisms of LDL oxidation.

2. Oxidized components of LDL

The process of LDL oxidation is assumed to occur in two main stages [3,7,23]. During the initial stages of LDL oxidation in vitro, oxidative modifications of LDL lipids can occur in the absence of changes or little change in apolipoprotein B100. Such modified LDL is called minimally oxidized LDL, which retains the affinity to the LDL receptor, has a little negative charge, activates anti-apoptotic signaling, and induces inflammatory changes with increased chemokines and cytokines (Fig. 1). The recruitment of inflammatory cells may result in a huge variety of enhanced cytokines and the continued oxidation of LDL. Subsequently, LDL lipids are further oxidized, and the LDL protein is also modified, leading to a loss of recognition by the LDL receptor and a shift to recognition by scavenger receptors (oxidized LDL receptors), leading to the development of macrophage foam cells, the hallmark of the artery lesion of fatty streak (Figs 1 and 2). This highly oxidized LDL is commonly termed oxidized LDL, but LDL undergoes various degrees of oxidation. Actually, such a diversity of LDL oxidation provides different biological effects of oxidized LDL to vascular cells [14,24–26]. For example, the tissue factor expression in endothelial cells is induced by mildly oxidized LDL but not by highly oxidized LDL. The lipids in highly oxidized LDL are cytotoxic and pro-apoptotic, whereas the mildly oxidized LDL is not, and stimulates the proliferation of smooth muscle cells and may contribute to prolongs of macrophage foam cells via a PI3 kinase/Akt-dependent mechanisms. In addition to the important roles of living macrophages in the development and progression of atherosclerotic lesion, macrophage death in early phase of atherosclerosis may contribute to decrease macrophage

Fig. 1. Mechanisms of LDL oxidation and macrophage foam cell formation in the artery wall. LDL that enters artery wall may be oxidized by vascular cells (endothelial cells, smooth muscle cells, and macrophages) with oxidizing enzyme including lipoxygenase and myeloperoxidase in the presence or absence of transition metal ions (iron or copper). Minimally oxidized LDL has a low affinity to macrophages scavenger receptors, and thereby, minimally oxidized LDL can be recycled into blood circulation and can be detected as a serum oxidized LDL. Such the minimally oxidized LDL stimulates adhesion molecules and chemokines. Extensively oxidized LDL can be taken up by macrophages through the scavenger receptors, leading to the formation of foam cells. These extensively oxidized LDL and minimally oxidized LDL enhance macrophage scavenger receptors with various modulations of cytokines.
burden and slow lesion progression, whereas macrophage death in late phase of atherosclerotic lesion may cause necrotic core formation of lesions, leading to the promotion of plaque rupture [7,27,28].

Core lipids of LDL particle are composed of cholesterol ester (CE) and triglyceride (TG), an outer monolayer is composed of free cholesterol (FC) and phospholipid (PL) including phosphatidylcholine (PC), and one molecule of apolipoprotein B100 surrounds the LDL particle [10]. Once free radical-mediated oxidation of unsaturated fatty acids occurs, a chain reaction leads to the formation of CE hydroperoxide (CEOOH) and PC hydroperoxide (PCOOH). Because cholesterol linoleate (CE18:2) is a major lipid of LDL, CE18:2 hydroperoxide (CE18:2-OOH), CE18:2 hydroxide (CE18:2-OH), and ketone (CE18:2=O) are mainly present in oxidized LDL [29,30]. Using negative ion chemical ionization gas chromatography (GC)/mass spectrometry (MS), F2-isoprostane is measured as an oxidation product of arachidonic acid [31]. A variety of the oxidation products of PL are identified and detected in the atherosclerotic lesion and oxidized LDL [32]. In addition, cholesterol is not so much susceptible to oxidation as unsaturated fatty acids, but the oxidation products, such as 7-ketocholesterol, are in occurrence.

In this way, the full spectrum of atherosclerotic lesion may be attributed to the biological properties of oxidized LDL. Such a contribution of oxidized LDL to the spectrum of atherosclerosis is presumably due to a variety of components of oxidized LDL in part because LDL could be oxidized by various mechanisms as described above in Introduction and as below.

3. Mechanisms of LDL oxidation

3.1. Metal ions

Lipid peroxidation leads to the formation of a broad array of different products with diverse biological activities including a variety of different aldehydes [33]. The primary products of lipid peroxidation, lipid hydroperoxides, undergo carbon–carbon bond cleavage via alkoxy radicals in the presence of transition metals forming short chain unesterified aldehydes [34]. Macrophage-mediated LDL oxidation enhanced formation of cholesteryl ester–core aldehydes of LDL [35]. Most of cells present in the arterial intima can promote LDL oxidation in vitro by its enzymes as shown in Fig. 1, which is cell-mediated LDL oxidation, but it arguably requires the presence of transition metals, microconcentration of iron or copper, in the culture medium, and metal chelators can block cell-mediated LDL oxidation [27,36–41]. Even in the absence of cells, high concentrations of free metal ions can oxidize LDL. Esterbauer et al established the method for continuous monitoring of in vitro oxidation of human LDL [42]. Briefly, after dialysis against phosphate buffer saline to remove EDTA at 4 °C, LDL (50–100 μg/mL) is oxidized with 1–10 μmol of copper at 37 °C, and conjugated diene formation during LDL oxidation is monitored by changes in wavelength absorbance at 234 nm spectrophotometrically (Fig. 3). In this way, metal ions are able to catalyze LDL oxidation in vitro.

Elevated levels of metal ions may be present in the advanced atherosclerotic lesions, but there is a paucity of quantitative information and the nature of metal ions. Tissue homogenates prepared from atherosclerotic lesions contain catalytically active metal ions, indicating that these metals may stimulate in vivo LDL oxidation in the artery wall, but it cannot be completely denied that redox metal ions are generated artificially during the process of tissue homogenization. Then, the results using a minimally invasive technique, electron paramagnetic resonance (EPR) spectroscopy and inductively coupled plasma mass spectrometry (ICPMS) were reported, showing that a direct correlation between iron levels and cholesterol accumulation in human artery samples from carotid endarterectomy [43]. However, hemochromatosis and Wilson disease increase levels of iron and copper in blood circulation and tissues, but patients with these diseases are not at increased risk for atherosclerosis [44]. Coronary heart disease is less extensive in patients with hemochromatosis, and epidemiological studies have shown that the relationship between tissue iron levels and atherosclerosis is controversial although in vitro studies with cultured cells have demonstrated that iron treatment can increase oxidative stress and oxidative susceptibility of LDL incubated with cells [45,46]. Haptoglobin is a pivotal protein for controlling the turn-over of iron and hemoglobin, and haptoglobin 2–2 genotype is associated with 2–5 fold increased risk of cardiovascular disease because of increased oxidative stress due to the inferior antioxidant protection [7,47,48]. In fact, vitamin E therapy can prevent cardiovascular events in diabetes patients with haptoglobin 2–2 genotype even when administered with statins. Although it shows indirect evidence, the relationship between iron and LDL oxidation might be suggested for humans. In addition, mouse model studies also have demonstrated the association of haptoglobin 2–2 genotype to increased active hemoglobin derived iron and the critical role of haptoglobin genotype in iron store, lipid peroxidation, and macrophage accumulation in atherosclerotic plaque [49,50].

There is increasing evidence that thiols may play an important role in LDL oxidation [40,51–53]. When LDL is incubated with iron at acidic pH, but not at pH 7.4, the formation of hydroperoxides and aldehydes...
is enhanced as cysteine concentration is increased. Cysteine may increase iron-mediated LDL oxidation by reducing Fe (III) to Fe (II) and reducing alpha-tocopherol radicals of oxidized LDL back to alpha-tocopherol [54,55]. The oxidation of cysteine to cystine may also generate oxygen- or sulfur-centered free radicals, which may oxidize LDL [54,56]. Atherosclerosis lesions are characteristic of a chronic inflammation and may be supposed to have a low pH in lesion sites. The media of arteries is known to be ischemic and the mid region of intimal atherosclerotic lesions may be ischemic as well [57,58]. Therefore, atherosclerotic lesion cells may use anaerobic glycolysis and then produce lactic acids, leading to acidification of the extracellular space. The extracellular space may also be acidified by macrophages and the greater effect can be achieved by activated macrophages as shown in human atherosclerotic lesions [59,60]. Although metal ions may be related to enhanced lipid peroxidation at low pH in atherosclerotic lesions, iron ions can accelerate LDL oxidation at low pH but copper ions make initial oxidation of LDL slower at low pH, while macrophage uptake of oxidized LDL is similar between iron- and copper-mediated oxidation [40]. The presence of free copper or iron in vivo is unlikely, and ceruloplasmin or transferrin is carrying those metal ions in blood, respectively. Ceruloplasmin, a 132-kDa protein that contains 7 copper atoms per molecule, was regarded as an antioxidant, but the potent oxidant activity of ceruloplasmin on LDL has been verified [61–63]. Hemin (globin degradation product), transferrin (holo form of iron binding protein), and ferritin may catalyze LDL oxidation [64–67]. In addition, acidic pH can make ceruloplasmin, copper-carrying protein, catalyze LDL oxidation more effectively, and also enables transferrin, iron-carrying protein, to catalyze LDL oxidation [67,68].

Tribble et al reported unique concepts concerning metal-dependent LDL oxidation [69]. The oxidative injury exclusive to LDL particle surface may process the biological properties of minimally oxidized LDL, which has low levels of oxidation products mainly composed of oxidized phospholipids. Such a minimally oxidized LDL is implicated in inflammatory process, cell proliferation and macrophage foam cell formation [3,10,14,24,26,53]. Iron (III) treatment can generate surface-oxidized LDL [63]. It may be that this full oxidation is mediated by copper or hemin. The oxidative injury to LDL core lipids is associated with a relative loss of function of minimally oxidized LDL and with a contrast increase in the biological activities as fully oxidized LDL, including enhanced uptake by macrophage scavenger receptors with marked changes in apolipoprotein B constituent. Minimally oxidized LDL still has the affinity to LDL receptor, but fully oxidized LDL is not able to bind LDL receptor [2,10,14]. Macrophages uptake oxidized LDL through scavenger receptors, but minimally oxidized LDL may avoid macrophage uptake because of low affinity to scavenger receptors (Figs. 1 and 2). Collectively, oxidative injury to LDL is initiated within the surface of LDL particle, and is subsequently transferred to the core of LDL, possibly in association with the accelerated phase of LDL lipid peroxidation.

3.2.1. Enzymes

3.2.1. Lipoxygenase

Lipoxygenase, non-heme iron-containing dioxygenase, is one of intracellular oxidation enzymes that directly oxygenate polyunsaturated fatty acids [18,70]. How intracellular lipoxygenase could promote LDL oxidation is of great interest (Fig. 1). In vitro, 15-lipoxygenase directly oxidizes LDL, and LDL exposed to fibroblasts transfected with the 15-lipoxygenase gene exhibited high levels of lipid peroxides [18,19,71]. In addition, the enzyme reaction products may seed LDL with hydroperoxides that subsequently decompose into reactive intermediates that promote further lipid peroxidation [12,17]. In vitro 15-lipoxygenase oxidizes LDL via both direct (enzymatic) and indirect (non-enzymatic) reactions, the latter of which produces radical oxidants that may contribute to the subsequent non-enzymatic lipid peroxidation [72]. Lipid peroxides generated by intracellular lipoxygenase could be released from cells and might translocate to LDL [18,53]. On the other hand, after treated with LDL, macrophage 12/15-lipoxygenase transmigrates from the cytoplasm to the membrane where it oxidizes LDL, and in this process the LDL oxidation may be dependent on LDL binding to macrophage LDL receptor-related protein (LRP) without subsequent endocytosis and degradation of LDL [73,74]. The oxidized cholesterol ester (cholesterol linolate) seems to be transported back to LDL particle, which might be also LRP-mediated [75]. However, the detail molecular mechanisms of LRP-mediated lipoxygenase translocation and LDL oxidation need further investigations. When LDL is exposed to 15-lipoxygenase in vitro, the major product is 13-S-hydroxyoctadecadienoic acid (13-S-HODE), a major hydroxide of linoleic acid [76]. In contrast, copper-mediated LDL oxidation generates similar amounts of 13-S-HODE and 13-R-HODE [76–78]. Namely, the elevated level of 13-S-HODE in atherosclerotic lesions may mimic the significant role of lipoxygenase in LDL oxidation in vivo.

In fact, elevated co-localization of mRNA and protein of 15-lipoxygenase with the epitope of oxidized LDL have been found in human atherosclerotic lesions, suggesting the possibility that the lipoxygenase promotes LDL oxidation and atherosclerosis [79,80]. However, compelling evidences for the relevance of lipoxygenase to atherosclerosis has been reported [7,44]. Unexpectedly, cholesterol-fed rabbits and Watanabe-heritable hyperlipidemia (WHHL) rabbits with macrophage overexpression of 15-lipoxygenase were protected against atherosclerosis [81]. In addition, the global knockout of 12/15-lipoxygenase increased rather than decreased atherosclerosis in apolipoprotein E-knockout mice [82]. By contrast, macrophage specific deficiency of 12/15-lipoxygenase in the apolipoprotein E deficient mice ameliorates atherosclerosis [83]. In addition, 12/15-lipoxygenase deficient mice, crossed with apolipoprotein E-knockout or LDL-receptor deficient mice, were remarkably resistant to atherosclerosis despite similar lipid levels and lipid profile with apolipoprotein E-knockout mice or LDL-receptor deficient mice [84–86]. Meanwhile, site-specific overexpression of 15-lipoxygenase in endothelium accelerated atherosclerosis in LDL receptor-deficient mice [87]. In the latter article, absence of 12/15-lipoxygenase decreased the ability of macrophages to form foam cells and to cause endothelial activation when exposed to native LDL, suggesting a critical role of 12/15-lipoxygenase in macrophage- and endothelial cell-mediated LDL oxidation. These compelling results indicate that 12/15-lipoxygenase may have both pro- and anti-inflammatory actions [88]. Namely, some level of lipoxygenase in vascular cells may be involved in atherogenesis, while some levels of lipoxygenase in global cells may exert anti-inflammatory functions [7]. This discrepancy in the functional relevance of 12/15-lipoxygenase to atherogenesis among animal models could be explained in part by the property differences in lipoxygenase isoforms in atherogenesis [7,88].

3.2.2. Myeloperoxidase

Myeloperoxidase (MPO), abundantly expressed in the azurophilic granules of leukocytes (neutrophils and monocytes), is an enzyme linked to inflammation and oxidative stress [89,90]. Neutrophile-released MPO catalyzes the formation of reactive substance species, including hypochlorous acid (HOCI) and has a role in host defense against pathogens. In other words, MPO, a heme protein secreted by granules of leukocytes (neutrophils and monocytes), is an enzyme linked to in

Figs. 1 and 2. Collectively, oxidative injury to LDL is initiated within the surface of LDL particle, and is subsequently transferred to the core of LDL, possibly in association with the accelerated phase of LDL lipid peroxidation.
l lipid peroxidation, oxidized LDL, advanced glycation end products, and nitrating species [17]. This system, which is independent of free metal ions, may be physiologically relevant because neutrophils and monocytes/macrophages generate H2O2 and chloride ubiquitously [53].

MPO has also been implicated in the development of atherosclerosis [91–94]. Some compelling evidences on the relevance of MPO to atherosclerosis were found in similar with lipoxigenase. MPO knockout in mice was expected to protect against atherosclerosis but in fact enhances atherosclerosis [95]. However, the over-expressed human MPO in LDL receptor deficient mice, fed a high fat/cholesterol diet, increased atherosclerosis about 2-fold, while MPO-knockout bone marrow-transplanted mice slightly promoted atherosclerosis [96]. Although conflicting results in the animal models with overexpression/knockout strategies of MPO, epidemiological studies have shown that higher concentrations of plasma MPO are associated with an increased risk of cardiovascular disease, independent of classical risk factors [23,44,90]. MPO-mediated oxidations need co-oxidants such as hydrogen peroxides or lipid peroxides. Thereby, overexpression or knockout of MPO might have little effects on oxidative stress. These studies also suggest that different pathways promote oxidation reactions in mouse and human atherosclerotic lesions.

The sources of MPO in the vascular wall are both local release from resident macrophages and transcytosis of intraluminally produced MPO by activated leukocytes [89,90]. Mitochondrial respiration, NADPH oxidase, xanthine oxidase, and nitric oxide synthase are major sources of highly reactive superoxide radicals, which are converted to hydrogen peroxide by superoxide dismutase. Hydrogen peroxides, less active than superoxide radicals, are also the cosubstrate for all MPO-catalyzed reactions. MPO can enhance the oxidative potential of hydrogen peroxide by producing a variety of reactive oxidants.

MPO is a highly cationic protein and capable of binding to endothelial cells, leukocytes and LDL. A number of reactive oxygen species generated by MPO oxidize lipid and protein of LDL, and the association of MPO and LDL may enhance LDL oxidation [96]. Many of MPO-catalyzed oxidation products are unstable and promote further oxidation of LDL, while a few stable products, modified tyrosine residues (3-chlorotyrosine and 3-nitrotyrosine) may serve as biomarkers of MPO-catalyzed oxidation.

3.3. Glycated LDL

Glycation of protein is a complex series of Maillard reaction. Early phase reactions are subjected to lysine and NH2-terminal amino acid residues, and late phase reactions form advanced glycation end products (AGEs). Actually, albumin is non-enzymatically glycated resulting from covalent binding of glucose to amino acids, and the principal site of glycation of albumin is also lysine residue basically at Lys-525 of NH2-terminal side [97–99]. The non-enzymatic glycation of LDL occurs in apolipoprotein B, lysine of which is the major amino acid undergoes glycation, making 2–17% of LDL-lysine glycated [100,101]. A higher proportion of lysine-bound glucose in apolipoprotein B, a higher content of fructosamine in LDL, and two-fold higher concentrations of serum glycated apolipoprotein B were found in patients with diabetes than in non-diabetic subjects [102–104].

Glycose enhances LDL lipid peroxidation by an oxidative pathway involving superoxide, and it raises the possibility that the chronic hyperglycemia of diabetes accelerates lipoprotein oxidation [105]. In fact, LDL from patients with diabetes is more susceptible to oxidation [106]. Increased evidences of small dense LDL, highly atherogenic presumably because of its susceptibility to oxidative modification, were found in patients with diabetes and hypertriglyceridemia [107–109]. Small dense LDL apolipoprotein B is more susceptible to glycation than buoyant LDL apolipoprotein B, and most of serum glycated apolipoprotein B is distributed in small dense LDL fraction [110].

Glycation and oxidation take place in vivo concurrently because free radicals can be generated by glycation itself from glucose and Amadori products, leading glycated LDL to the enhanced susceptibility to further oxidation [111]. However, oxidized LDL may be generated during glycation, but oxidized LDL is not necessarily glycated in part because vitamin E and transition metal ion chelation can inhibit LDL oxidation while permit LDL glycation [112,113]. In vitro oxidation with copper is also enhanced in LDL from patients with diabetes, depending on glycation and increased ration of esterified/free cholesterol, which can provide more fatty acids, supporting a greater potential for lipid peroxidation [114].

A certain levels of LDL oxidation may be inevitable during glycation, but more extensive oxidation presumably requires supplementary oxygen free radicals [115]. LDL glycation may predominate over LDL oxidation in vivo [116].

3.4. Nitric oxide and oxidation mechanism

Nitric oxide (NO), a more powerful antioxidant than vitamin E, is basically able to inhibit lipid peroxidation, and NO is a stable radical that fails to oxidize LDL at physiological pH but NO is rapidly inactivated by superoxide anion to form peroxynitrite (ONOO−), a potent oxidant [117–120]. Therefore, NO plays a pro-oxidant role when present simultaneously with superoxide anion, which is implicated in the mechanisms of LDL oxidation. Endothelial cells, smooth muscle cells, and macrophages generate superoxide anion, and thereby peroxynitrite or other reactive nitrogen intermediates could be formed in the artery wall and lead in part to cell-mediated LDL oxidation [121]. Endothelial cells are able to oxidize LDL when NO bioavailability is low, and by contrast, LDL oxidation may be inhibited if NO released by endothelial cells is elevated [122–124]. NO production by vascular cells and its diffusion into LDL particles may provide a key mechanism of antioxidant at the hydrophobic core of LDL where the ratio of oxidizable lipids to endogenous antioxidants is greater than at the surface of LDL particle [125]. Therefore, NO is an essentially antioxidant, generated by neutrophils and endothelial cells which can also release superoxide anion. Both NO and superoxide anion react slowly with other molecules (iron or ascorbate), and thereby they easily react each together to form peroxynitrite [119,124]. The formation of NO and superoxide anion occur concurrently in endothelial cells, and consequently peroxynitrite is generated within vessel walls in response to some endothelial cell stimulations. The reaction of NO with superoxide anion under H+ forms peroxynitrous acid (ONOOH), which subsequently is decomposed to nitric oxide radical and hydroxyl radical [124,126]. These radicals are potent oxidant species which may initiate and propagate lipid peroxidation through their reactions with unsaturated fatty acids. In this context, the concurrent formation of NO and superoxide anion provokes the generation of peroxynitrite and lipid peroxidation. Peroxynitrite in turn reacts with tyrosine to form 3-nitrotyrosine, a stable product. Alternatively, MPO is also involved in the generation of reactive nitrogen species [127,128]. When L-tyrosine is incubated with MPO, hydrogen peroxide, nitrite, and 3-nitrotyrosine are formed.

Taken together, both pro- and anti-oxidant effects of NO have been proposed. Changes in the balance of NO and superoxide may be significantly implicated in lipid and LDL peroxidation, because superoxide may both remove anti-atherogenic action of NO and provide pro-atherogenic action of peroxynitrite.

4. Conclusive remarks

As described above, the mechanisms underlying LDL oxidation in vitro and in vivo are wide-ranging and complicated. Xanthine oxidase, present at high concentrations in endothelial cells of capillaries, is also
assumed to be associated with lipid peroxidation mechanism, and exists as dehydrogenase and oxidase of which the former is predominant [17,23,129]. Only the latter generates superoxide anion, but the precise regulation of endothelial xanthine oxidase is not clear. In clinical settings, LDL from patients with diabetes and/or renal insufficiency is susceptible to oxidative modification [106,130]. The host defense to infection and inflammation induces LDL oxidation in vivo, and the increased LDL oxidation may promote atherogenesis in patients with infection and inflammation [131].

Recently, carboxylated LDL common in patients with renal insufficiency is focused on roles in the disease development similarly to glycoxidized LDL present in diabetes patients [132]. Protein carboxylation is catalyzed by MPO, leukocyte heme peroxidase, and inflammation-driven protein carboxylation is dominantly involved in the mechanisms of carboxylation in atherosclerotic lesions where oxidized LDL plays relevant roles. The underlying mechanism of MPO-catalyzed protein carboxylation is MPO/SCN\(^{-}\) (electrophilic species of thiocyanate) system, where OCN\(^{-}\) (electrophilic species cytanate) is formed in the presence of catalyst, MPO. The reaction of OCN\(^{-}\) with e-amino groups of protein lysine residues produces e-carboxyllysine, known as homocitruline. The carboxylated LDL as well as uremic LDL has been reported to provide proatherosclerotic effects, including diminished affinity to LDL receptor, increased proliferation of smooth muscle cells, and enhanced apoptosis of endothelial cells in a similar way with oxidized LDL. However, it is quite intriguing that carboxylated LDL can bind scavenger receptor A1 but not CD36, while oxidized LDL can bind both scavenger receptors. Carboxylated LDL is similar to oxidized LDL, but it remains to be cleared whether LDL carboxylation is one of mechanisms underlying LDL oxidation.

LDL oxidation could be an obligatory step in vivo in the pathogenesis of atherosclerosis and vascular diseases. However, no one knows well where and how it occurs, which enzymes are essentially responsible for it. The underlying mechanisms of LDL oxidation must be further explored to reveal appropriate ways for the diagnosis and treatment of atherosclerosis and its relevant diseases.

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References


