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Oxidative Stress in Atherosclerosis

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Abstract

Purpose of Review Atherosclerosis is now considered a chronic inflammatory disease. Oxidative stress induced by generation of excess reactive oxygen species has emerged as a critical, final common mechanism in atherosclerosis. Reactive oxygen species (ROS) are a group of small reactive molecules that play critical roles in the regulation of various cell functions and biological processes. Although essential for vascular homeostasis, uncontrolled production of ROS is implicated in vascular injury. Endogenous anti-oxidants function as checkpoints to avoid these untoward consequences of ROS, and an imbalance in the oxidant/anti-oxidant mechanisms leads to a state of oxidative stress. In this review, we discuss the role of ROS and anti-oxidant mechanisms in the development and progression of atherosclerosis, the role of oxidized low-density lipoprotein cholesterol, and highlight potential anti-oxidant therapeutic strategies relevant to atherosclerosis.

Recent Findings There is growing evidence on how traditional risk factors translate into oxidative stress and contribute to atherosclerosis. Clinical trials evaluating anti-oxidant supplements had failed to improve atherosclerosis. Current studies focus on newer ROS scavengers that specifically target mitochondrial ROS, newer nanotechnology-based drug delivery systems, gene therapies, and anti-miRNAs. Synthetic LOX-1 modulators that inhibit the effects of Ox-LDL are currently in development.

Summary Research over the past few decades has led to identification of multiple ROS generating systems that could potentially be modulated in atherosclerosis. Therapeutic approaches currently being used for atheroslcerotic vascular disease such as aspirin, statins, and renin-angiotensin system inhibitors exert a pleiotropic antioxidative effects. There is ongoing research to identify novel therapeutic modalities to selectively target oxidative stress in atherosclerosis.

Keywords Oxidative stress · Atherosclerosis · LOX-1 · OxLDL · Anti-oxidants · Reactive oxygen species

Introduction

Atherosclerosis is a chronic inflammatory disease characterized by accumulation of lipids and inflammatory cells in the walls of medium and large-sized arteries [1]. The pathogenesis of atherosclerosis involves activation of pro-inflammatory signaling pathways, expression of cytokine/chemokine, and increased oxidative stress. Oxidative stress is the imbalance in favor of increased generation of reactive oxygen species (ROS) and/or reduced body's innate anti-oxidant defense systems [2]. ROS play an important role in inflammatory responses, apoptosis, cell growth, and alteration in vascular tone as well as in oxidation of LDL-cholesterol, which is thought to be more important that native-LDL in atherogenesis [3]. ROS production in the vessel wall is increased in all conditions considered risk factors for atherosclerotic cardiovascular disease (CVD) such as hypertension, diabetes, smoking, and dyslipidemia [4••]. In this review, we discuss the role of ROS and anti-oxidant pathways in the development and progression of atherosclerosis.

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Mechanisms of Atherogenesis

The initial event in the development of atherosclerosis is endothelial injury. This causes infiltration into and accumulation of low-density lipoprotein cholesterol (LDL) in the sub-endothelial space. LDL becomes oxidized to form oxidized LDL (ox-LDL) in pathologic states [5]. The modified lipoprotein particles increase the expression of cell adhesion molecules (like vascular cell adhesion molecules-1 [VCAM-1], P and E- selectins) on the endothelial cells leading to leukocyte recruitment (mainly monocytes and T-lymphocytes) into the sub-endothelial space. With the interplay of chemoattractant proteins like monocyte chemoattractant proteins (MCP-1), eotaxin and interferon (INF)-γ, these inflammatory cells migrate into the intima. The monocytes differentiate into macrophages, express scavenger receptors (SRs) (such as CD 36, SRA, and LOX-1), and internalize modified lipoproteins. These lipid laden macrophages are called foam cells due to their appearance, and their presence in the arterial wall is a hallmark of early atherosclerotic lesion. T-lymphocytes and mast cells that undergo migration into the intima, along with foam cells, release a variety of cytokines that promote inflammation and ROS generation. Growth factors released by these cells as well as ROS stimulate smooth muscle cell migration and collagen deposition leading to the development of an atheromatous plaque.

Importantly, ROS induce the expression of SRs in smooth muscle cells and their transformation into foam cells. ROS also induce release of matrix metalloproteinases (MMPs) that degrade the fibrous wall of the atheromatous plaque and basement membrane of the endothelial cells resulting in physical disruption of the plaque (Fig. 1). Physical disruption of the plaque can occur by superficial erosion of the endothelial cells due to damage of their basement membrane, disruption of micro-vessels in the plaque causing micro-hemorrhage/thrombosis, or disruption of the fibrous cap exposing the pro-thrombogenic content of the plaque. They cause sudden expansion of these lesions and may trigger thrombosis in the arteries hosting them. These physical disruptions may not always cause pathologic obstruction to blood flow. Many times, anti-coagulant pathways override and a healing process takes over. This leads to further smooth muscle proliferation and accumulation of collagen thereby transforming a fatty atherosclerotic plaque to a more fibrous plaque [6].

Endogenous ROS Generating Systems in Atherosclerosis

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidase, mitochondrial enzymes, lipoxygenases, myeloperoxidases, and uncoupled endothelial NO synthase (eNOS) are the major ROS generators in the blood vessels [4••].

NADPH oxidases (NOX) are perhaps the most important ROS generating system in the cardiovascular system. Their activation results in the generation of superoxide anion from oxygen molecule by transferring electron from NADPH. They are multi-subunit enzyme complexes, with two membrane bound units—$gp91^{phox}$ and $p22^{phox}$. Various isoforms of NOX have been studied for their role in atherosclerosis [7]. In mice, Nox1 and Nox4 are expressed by VSMCs and Nox2 and Nox4 by endothelial cells [8–10]. Nox 1 is upregulated in diabetic state, and its deletion in ApoE-KO mice reduces atherosclerosis. Similarly, Nox 2 deletion has also been shown to be related to reduction in atherosclerosis in descending aorta in mouse models. The role of Nox4 in atherogenesis is controversial, and some recent studies show it to be protective against atherogenesis. This is likely due to generation of hydrogen peroxide by Nox4 rather than superoxide and its inability to interact with NO. [11] Nox 5 which is absent in the mouse genome was found to be upregulated in diabetes, hypertension, and human atherosclerotic lesions [4••, 12–14]. NOX activity in macrophages is an important component in the generation of ox-LDL. NOX activity is thought to play a role in the expression of endothelial adhesion molecules, monocyte infiltration, and VSMC proliferation [15].

Xanthine oxidases are found in endothelial cells and in plasma and generate superoxide anions and hydrogen peroxide. Their levels have been found to be increased in human atherosclerotic plaque [16]. Angiotensin II and oscillatory shear stress increase the expression of endothelial xanthine oxidases [17, 18]. Studies have shown that xanthine oxidase inhibitors can reduce atherosclerosis development in ApoE-KO mice and mitigate endothelial dysfunction in heavy smokers [19, 20].

Xanthine oxidase stimulates LOX-1 and CD-36 expression on macrophages and VSMCs and increases ROS generation. This in turn activates NLRP3 inflammasome and downstream inflammatory mediators causing transformation of macrophages and VSMCs into foam cells. In addition, uric acid generated by xanthine oxidase triggers foam cell formation through the expression of CD-36 [21]. A recent population-based study suggests that allopurinol may have a role in reducing the risk of coronary artery disease [22]. However, further research is needed to establish its role in human atherosclerosis.

Mitochondrial enzymes produce superoxide anions at physiological levels and can become pathologic due to mitochondrial dysfunction leading to excess ROS production or due to failure of antioxidant mechanisms. Accelerated atherosclerosis and elevated mitochondrial ROS are seen in experiments involving the deletion of anti-oxidant systems in ApoE-KO mice, suggesting a role for mitochondrial ROS in atherogenesis [23]. There is a cross talk between the NOX

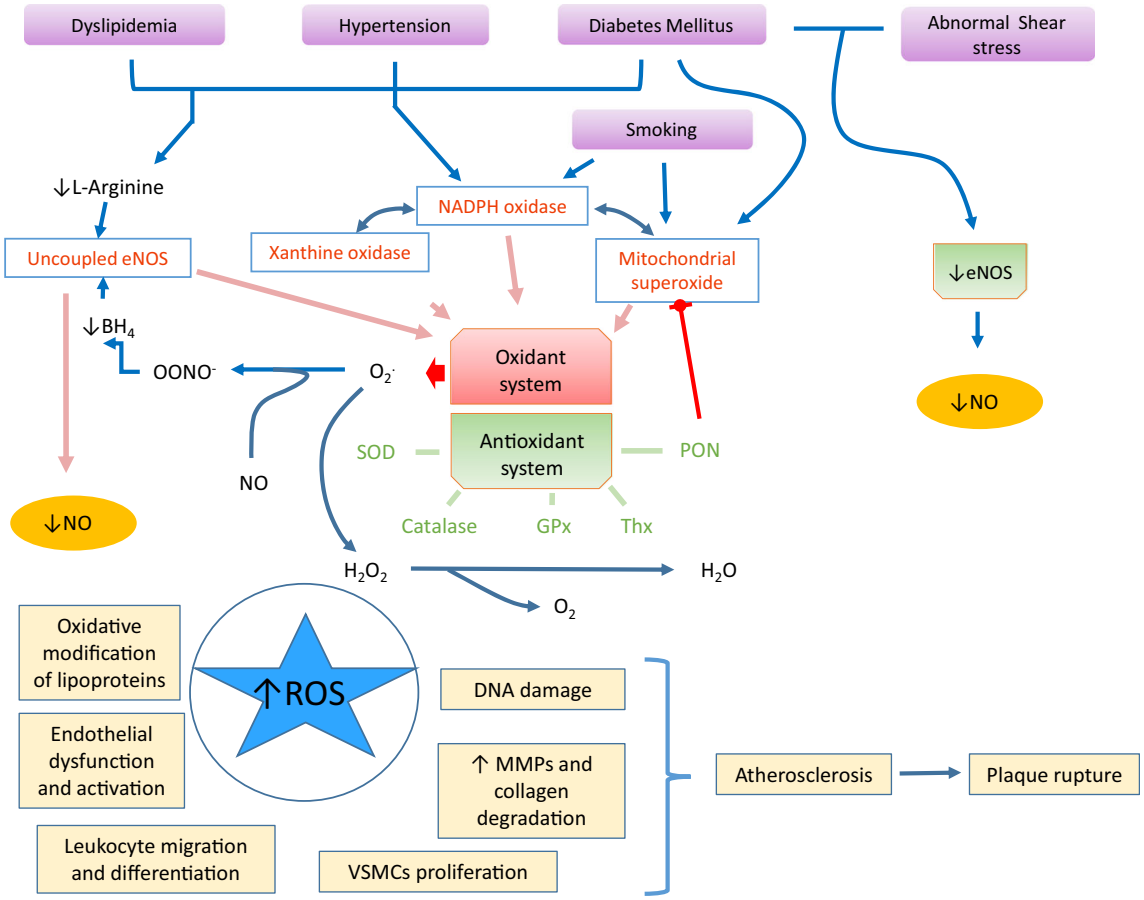

Fig. 1 Postulated mechanisms by which cardiovascular risk factors affect ROS generation and endothelial function along the interplay of oxidant and antioxidant systems, in generation of ROS. Further, the effect of ROS on various stages of atherosclerosis is also depicted

system and the mitochondrial ROS system in terms of ROS generation and endothelial dysfunction which may have relevance in atherogenesis.

Lipoxygenases generate hydroperoxidases using arachidonic acid as their substrate. 5-Lipoxygenase and 12/15-lipoxygenases are the types which are related to atherogenesis [24]. They induce NOX activation in VSMCs resulting in oxidative stress in vascular tissues. Leukotrienes which are the end products of lipoxygenases are themselves pro-inflammatory and activate endothelial cells, macrophages, and foam cells. They also lead to release of cytokines and MMPs [25]. 5-Lipoxygenase inhibition improves post-inflammatory changes after myocardial ischemia [26]. 12/15-lipoxygenase is mainly involved in oxidative stress related to diabetic cardiomyopathy and is currently being investigated as a potential therapeutic target [27].

Myeloperoxidase is mainly found in neutrophils and to a small degree in monocytes and generates hypohalous acids such as hypochlorous acid ($HOCl^-$) from reaction with H_2O_2. It has bactericidal effect and plays an important role in immune system. Dysfunction of this system generates ROS and induces oxidative stress [24]. Excess levels of thiocyanate (e.g., from smoking) are converted to hypothiocyanous acid by myeloperoxidase [28]. This can lead to altered MAPK signaling and apoptosis in vascular cells. In addition, myeloperoxidase oxidizes LDL, HDL, and NO, thereby triggering and enhancing atherogenesis [29–31]. Of note, myeloperoxidase levels are increased in patients with atherosclerotic coronary artery disease [32].

NO plays a vasoprotective role and is constitutively expressed in endothelium by activation of eNOS. Under conditions of oxidative stress, eNOS becomes dysfunctional. Superoxide anions generated by the ROS systems combine with NO to form a highly reactive $OONO^-$ (Fig. 1). It combines with eNOS cofactors (such as tetrahydrobiopterin [BH4]) and uncouples oxygen reduction from NO synthesis. Uncoupled eNOS produces superoxide anions instead of NO and hence is a ROS generator. Studies have shown an inverse relationship between availability of cofactors of eNOS and development of endothelial dysfunction [33, 34].

Anti-Oxidant Systems in Atherosclerosis

The major anti-oxidant systems in the vascular wall are the super oxide dismutases (SOD), glutathione peroxidases, catalases, paraoxonases (PON), thioredoxins, and

NO [4••]. SOD converts superoxide to hydrogen peroxide which is further degraded by glutathione peroxidases, catalases, and thioredoxins [35]. SOD consist of three isoforms—SOD1 (cytoplasm and inner mitochondrial membrane), SOD2 (mitochondrial matrix), and SOD3 (extracellular) based on the location where it is found. Upregulation of SOD does not translate directly to reduced ROS generation. This is due to the increased amount of distal oxidants produced by its end product, hydrogen peroxide, which can favor atherosclerosis. Overexpression of catalase which can degrade hydrogen peroxide, in addition to SOD1, in ApoE-KO mice reduces atherosclerosis; however, SOD1 overexpression alone may increase the extent of atherosclerosis [36]. Hence, although SOD may reduce superoxide anion-mediated damages, it can enhance oxidative stress if there are insufficient enzymes downstream the pathway to detoxify its end product [4••].

Catalases convert hydrogen peroxide to water and oxygen. They are found in peroxisomes and improve atherosclerosis in high-fat diet mice models [36]. Based on the type of atherosclerotic models studied, the anti-oxidant enzymes have shown variable results regarding the development of atherosclerosis. It is thought that atherogenic stimuli present in Apo-E KO mice are largely due to accumulation of peroxides. This state of peroxide accumulation is ameliorated, at least in part, by catalases.

Glutathione peroxidases are anti-oxidant enzymes that are found in many cells and can reduce a variety of peroxidases including lipid peroxidases and oxidized phospholipids. In mouse peritoneal macrophages, glutathione peroxidase deficiency increases ox-LDL-induced foam cell formation and proliferation of macrophages [37]. In this study, overexpression of glutathione peroxidase in Apo E-KO mice decreased pro-atherogenic events such as expression of adhesion molecules in endothelial and monocytes. Further, there was evidence of decreased lipid peroxidation and decreased sensitivity of vascular cells to oxidized lipids [38].

Paraoxonases (PON) are a family of proteins (consisting of PON 1, PON 2, PON 3), which reduce oxidative stress, decrease lipid peroxidation, and diminish atherosclerosis in animal models [4••]. Paraoxonase 1 is found associated with HDL, and its upregulation in Apo E-KO mice protects against lipid oxidation and reduces in atherosclerosis [39]. Paraoxnase 2 is expressed on the vessel walls and resides in the membranes of ER or mitochondria and reduces superoxide formation. It can translocate to plasma membrane in response to lipid peroxidation and was shown to reduce oxidative stress and atherosclerosis in mice model of atherosclerosis [40]. Similarly, paraoxonases 3 prevents mitochondrial superoxide formation, and its expression was found to be low in the VSMCs of atherosclerotic plaques suggesting its protective role [41]. PON3 knockout mice were found to have a 60% increase in atherosclerotic lesion size when fed with a cholate-cholesterol diet [42].

Thioredoxins (Trx) system can reduce hydrogen peroxide and other target proteins. Downregulation of Trx1 (cytosolic) suppresses VCAM-1 and ICAM-1 expression and prevents initiation of atherosclerosis [43]. Suppression of mitochondrial Trx2 in endothelial cells causes endothelial dysfunction and initiates a prothrombotic phenotype in mouse models [44].

Nitric oxide synthases (NOS) play both an antioxidant and pro-oxidant role in atherosclerosis. Endothelial nitric oxide (eNOS) is constitutively expressed in endothelial cells. NO produced by eNOS activation inhibits LDL oxidation, leukocyte adhesion and migration, VSMC proliferation, and platelet aggregation [45]. eNOS deletion in Apo-E KO mice increases atherosclerosis [46]. Neuronal NO synthase (nNOS), though constitutively expressed in central and peripheral nerve cells, is also found in vascular wall that contributes to vasodilation and is considered anti-atherogenic. On the other hand, inducible NOS (iNOS) which is induced during inflammation, oxidative stress, and sepsis is pro-atherogenic. It is likely due to the formation of peroxynitrite by iNOS which is a pro-atherosclerosis oxidant [47]. In addition, iNOS activation can lead to relative deficiency of BH4, for eNOS, thereby uncoupling eNOS. Uncoupled eNOS is a ROS generator and contributes to atherogenesis.

Risk Factors for Atherosclerosis and Oxidant Stress

There is a state of oxidant stress in almost all risk factors for cardiovascular disease (Fig. 1). Hypertension, diabetes, smoking, and dyslipidemia activate the NADPH oxidase system resulting in excess generation of superoxide anions [48–50]. These conditions also lead to L-arginine and BH_4 deficiency causing uncoupling of eNOS, endothelial dysfunction, and further ROS generation [51]. Hyperglycemia stimulates superoxide anion generation from the mitochondrial electron transport chain, leading to production of advanced glycation end products (AGEs) which can activate NADPH oxidases, inhibit eNOS, and activate LOX-1 [50]. Smoking is a potent activator of NADPH oxidase at the endothelial level [52]. In addition, smoking directly oxidizes LDL. Ox-LDL activates NADPH oxidases more than the native-LDL and decreases eNOS activity [49]. Oscillatory shear stress on vessel walls decreases eNOS expression and promotes ROS generation via NADPH oxidase activation [53, 54]. The precise mechanism by which ROS and NO regulate LDL uptake in arterial walls is still under

investigation. Both, however, lead to oxidation of various phospholipids and generation of ox-LDL.

Role of Ox-LDL and LOX-1 in Atherosclerosis

It is now generally accepted that ox-LDL plays a more important role than n-LDL in athrogenic process. Several studies have shown elevated plasma levels of ox-LDL in patients with atherosclerotic CVD [55]. LDL oxidation can occur as a result of ROS generation at the level of the arterial wall [45]. Various studies have shown that ox-LDL promotes ROS generation in endothelial cells, VSMCs, and macrophages. It also inhibits eNOS activity in endothelial cells. The SRs for ox-LDL facilitate binding of modified lipids, self-proteins, and pathogenic organisms. Several classes (class A–class H) of SRs have been identified. Among them, SR-A type I and II, cluster of differentiation 36 (CD36), and LOX-1 are thought to play an important role in the uptake of ox-LDL and formation of foam cells (Fig. 2). Although CD36 and SR-A contribute to 75–90% of ox-LDL uptake, their role in promoting atherogenesis is controversial [56]. On the other hand, LOX-1 is thought to play an important role in atherogenesis, and its deletion has been shown to reduce atherogenesis in LDLr-null mice [57]. LOX-1 expression is upregulated by pro-inflammatory cytokines, angiotensin II, modified lipoproteins, hyperglycemia, AGEs, and free radicals. Of note, LOX-1 is also upregulated in pro-atherogenic states such as hypertension and diabetes [58].

LOX-1 and Endothelial Cells

LOX-1, a lectin-like receptor, is the major receptor for ox-LDL in endothelial cells involved in the uptake of ox-LDL [59]. It is involved in the transcription of nuclear factor (NF)-κB which in turn increases the expression of adhesion molecules in the endothelial cells [60]. It also increases the expression of monocyte chemotactic protein 1 (MCP-1) which helps in the recruitment of monocytes, an early step in atherogenesis [61]. LOX-1 activation induces apoptosis of endothelial cells. It causes downregulation of anti-apoptotic, Bcl-2, and neuronal inhibitory apoptotic protein and upregulation of caspase 3 and caspase 9 which cleave the anti-apoptotic proteins [62]. It decreases the transcription of eNOS resulting in a decrease in NO production which induces a state of vasoconstriction. LOX-1-mediated arginase II activation in the endothelial cells causes down-regulation of eNOS and, thereby, loss of vasodilator potential. Arginase II is involved in the regulation of arginine/ornithine concentration in the cell and, hence,

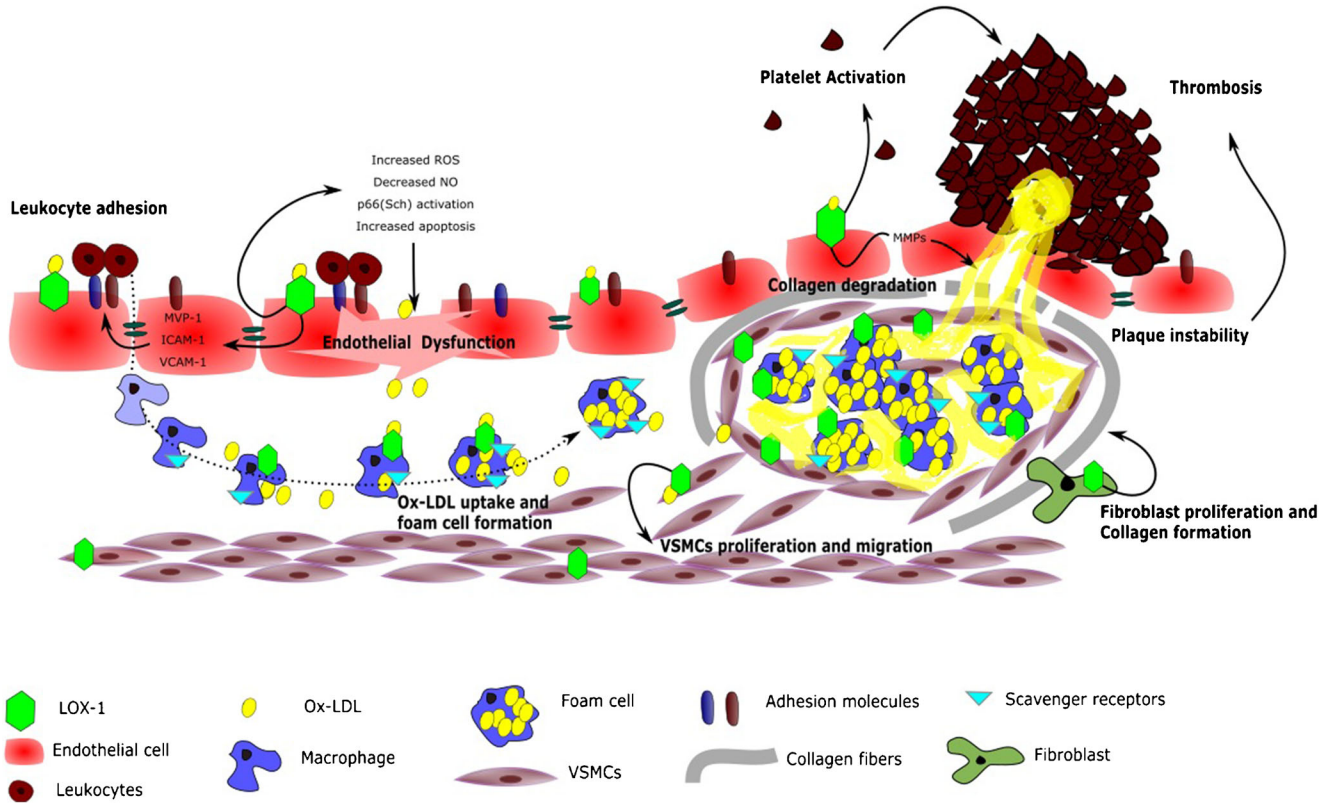

Fig. 2 Role of Ox-LDL and LOX-1 in atherogenesis and thrombosis

compete for the common substrate L-arginine thereby downregulating eNOS [63]. Recent studies have shown that LOX-1 mediates activation of adaptor protein-p66(Shc) [64]. P66(Shc) is involved in endothelial dysfunction seen in hypertension, in response to increased cyclic stretch of vessel walls. P66(Shc) are thought to increase ROS generation and reduce NO availability leading to vascular dysfunction [65].

LOX-1 and Macrophages

Compared to other SRs, LOX-1 in non-stimulated macrophages accounts for only 5–10% of the ox-LDL uptake, but it can rise up to 40% in macrophages when LOX-1 is upregulated [66]. Exposure to pro-inflammatory cytokines, high glucose concentration, ox-LDL, and lypophoshatidylcholine causes upregulation of LOX-1 and suppression of other scavenger receptors [58]. Hence, it is thought that, in atherosclerotic lesions which are growing in a pro-inflammatory environment, LOX-1 plays a major role in the uptake of ox-LDL and foam cell formation by macrophages [67]. LOX-1 also is very prominent in unstable atherosclerotic plaques with intense apoptosis [58].

LOX-1 and VSMCs

VSMC proliferation and migration are an important step in intimal hyperplasia of atherosclerosis. Role of LOX-1 in intimal hyperplasia following balloon injury has been studied in rat models. LOX-1 antibody was able to significantly suppress intimal hyperplasia, oxidative stress, and leukocyte infiltration compared to the control group after balloon injury on medium sized arteries [68]. Further evidence came from studies in LDLR knockout mice. Deletion of LOX-1 gene in ApoE$^{-/-}$ mice significantly decreased the proliferation and migration of VSMCs and luminal thickness. In addition, it reduced collagen deposition and pro-inflammatory and pro-oxidant signals [57, 69].

Role of LOX-1 in Plaque Instability and Platelet Activation

In addition to its effect on various cells involved in atherosclerosis, LOX-1 modifies plaque stability and activates platelets which are critical in the development of acute coronary syndromes. Ox-LDL through LOX-1 contributes to increased MMP-1, MMP-2, MMP-3, and MMP-9 activity [69, 70]. ox-LDL-treated human aortic endothelial cells show increased expression of LOX-1 and production of MMP-9. Treatment with antioxidants or anti-LOX-1 antibodies negates these effects [71]. Also, elevated concentrations of ox-LDL induce apoptosis in VSMCs. In contrast to normal LDL, ox-LDL causes sustained elevation in ROS production for up to 45 min [72]. ox-LDL upregulates LOX-1 in the VSMCs and is thought to trigger apoptosis [73]. Enhanced MMP production and apoptosis of VSMCs contribute to plaque instability. As mentioned above, LOX-1 expression is very prominent in unstable atherosclerotic plaques which intense apoptosis [58].

LOX-1 is involved in activation of platelets in multiple ways. LOX-1 is expressed on the activated platelets. Its binding to ox-LDL can be inactivated by anti-LOX-1 antibody. It is involved in ADP-induced platelet aggregation and activation of fibrinogen receptors on platelets which are critical components in platelet clumping [74]. Platelets can also adhere to the endothelial LOX-1 receptors, interact with LOX-1 and CD 40, and induce release of endothelin-1 [75]. In addition, platelet-endothelial interaction leads to increased superoxide production by endothelial cells causing inactivation of NO resulting in endothelial dysfunction [76].

Genetic Variants in Oxidative Stress Pathway Genes

Multiple gene polymorphisms in the oxidative stress pathway genes identified were found to modify the risk of atherosclerosis. C242T and A640G polymorphisms of the CYBA gene that encodes P22-phox of NADPH oxidase are among those that are extensively studied. These polymorphisms were shown to decrease ROS generation by NADPH oxidase [77]. However, multiple trials yielded conflicting results with regards to its effects on CAD risk. A recent meta-analysis reported a significant decreased risk of CAD in A640G polymorphism and in Asian population, with C242T polymorphism [78]. Among the SOD enzymes, 3 allele variants of the SOD1 gene (rs9974610, rs10432782, rs1041740) in type 2 diabetics were related to increased death from cardiovascular causes [79]. Polymorphisms in SOD2 resulting in A16V replacement in mitochondrial targeting domain have resulted in an increase in CAD risk and myocardial infarction [80]. Similarly, function variants of SOD3 enzymes (R213G) have been linked to elevated cardiovascular risk [81]. Glutathione peroxidase polymorphism resulting from a Pro198Leu replacement leading to modification of its enzyme activity has been found to be associated with increased cardiotid intima-media thickness and increased CAD risk [82]. However, conflicting reports from other studies exist, which showed its lack of association with coronary stenosis and stroke [83, 84]. Further, multiple studies have suggested association of eNOS variants (Glu298Asp, Intron 4) with CAD risk. Though genetic variants in the oxidative stress pathways have been shown to significantly influence atherosclerosis, there are many conflicting reports and further research is needed before definite conclusions can be made [4••].

Table 1 Effect of various pharmacological agents on oxidative stress

Drugs	Effect on ROS generators	Effect on anti-oxidant system	References
ACE inhibitors and ARBs	Decrease NOX, XO, and mitochondrial superoxide Prevent eNOS uncoupling	Increase BH_4 Increase SOD3	[85–88]
Aspirin	Inhibits ox-LDL mediated LOX-1 expression and ROS generation	Decrease NOX-4 and iNOS Decrease MMP-1 activity	[89, 90]
Statins	Decrease NOX, Prevent eNOS uncoupling	Increase SOD1, SOD3 and GPx Increase BH_4 Increase eNOS expression and activity	[91–94]
PETN	Decrease cardiac NOX Decrease Serum XO Decrease eNOS uncoupling	Increase BH_4 Increase SOD	[95, 96]
Resveratrol	Decrease NOX, mitochondrial superoxide Prevent eNOS uncoupling	Increase BH_4 Enchance eNOS expression and actitivy Induce SOD, GPx	[97–100]
Nebivolol	Inhibit NOX Prevent eNOS uncoupling	Increases eNOS	[101–103]
MitoQ	Decrease mitochondrial superoxide		[104]

Therapeutic Strategies in Reducing Oxidative Stress

Many pharmacologic agents that are currently in use modulate oxidative stress and improve atherogenesis. They reduce ROS generating systems and improve the anti-oxidant mechanisms (Table 1) [35]. Clinical studies designed to study the effects of traditional antioxidant supplements mostly failed in improving CV event rate in moderate to high-risk patients. It is thought that the concentration of antioxidants at the cellular level is not adequate to produce any significant effect, or the anti-oxidant therapy was started late in the course of disease. Small amounts of oxidants can initiate gene for antioxidant production at cellular level and that may nullify the effects of routine antioxidant use. There is lack of clinical studies evaluating the role for mitochondrial ROS which may play a pivotal role in atherosclerosis. Novel ROS scavengers that target mitochondrial ROS—such as mitoquinone which has a mitochondrial targeted quinine moiety—are being studied currently. They diminish free radical formation without affecting mitochondrial oxidative phosphorylation. They have been shown to reduce macrophage content and cell proliferation within plaques and inhibit multiple features of metabolic syndrome in mice models [104, 105•].

Drug delivery systems using nanotechnology are being investigated to improve endothelial function. Lipid-in-water nano-emulsions have been shown capable to selectively deliver 17-β estradiol into the atherosclerotic plaque to improve NO production, decrease gene expression of pro-inflammatory molecules, and reduce size of lesion [106•].

Gene therapies targeting overexpression of antioxidant systems have been studied in animal models [107]. Overexpression of SOD3 was shown to reduce ROS levels and inhibits in-stent restenosis in rabbit aorta. Catalase overexpression was shown to improve atherogenesis in ApoE null mice [36]. Further, targeted over expressions of genes involved in increasing BH_4 production may reduce eNOS uncoupling and thereby superoxide production [108].

MiRNA is a small non-coding molecule that control gene expression by exerting post-transcription effects. MiRNAs play a role in increasing inflammation, oxidative stress, apoptosis, and angiogenesis in the vessel wall. Thus, they are potential therapeutic targets. Viral vectors or nanoparticles can be made to deliver siRNAs, leading to silencing of molecules involved in endothelial activation and adhesion thereby improving CVD [109•, 110]. Anti-microRNA oligonucleotides and antisense oligonucleotides can also be used to modulate gene expression. Mipomersen is an FDA-approved antisense oligonucleotides which is used in familial hypercholesterolemia that targets ApoB. Further studies regarding modulation of gene expression are ongoing.

LOX-1 which plays a significant role in atherogenesis is a favored therapeutic target by many researchers. Various naturally occurring compounds like gingko biloba, flavonoids, and curcumin modulate atherosclerosis through LOX-1-mediated pathways. Synthetic LOX-1 modulators are being developed to prevent oxLDL-LOX-1 interaction. In addition, development of antibodies to LOX-1 by exposing chicken to recombinant human LOX-1 is in progress. Initial animal studies involving LOX-1 inhibition to reduce atherosclerosis appear to be promising [111••].

Conclusion

Oxidative stress and inflammation are major features in the development of atherosclerosis. Atherosclerosis risk factors are associated with excess ROS generation and oxidation of LDL. Ox-LDL acting on cell types promotes atherogenesis.

Therapeutic strategies targeting ROS generation, antioxidant systems, and inhibition of formation of ox-LDL and blockade of its receptors may prevent oxidative stress and improve atherosclerosis. Many drugs such as aspirin, statins, and renin-angiotensin system inhibitors, which are already in clinical use, exert a pleiotropic antioxidative effects. There is ongoing research to identify newer therapeutic modalities to selectively target oxidative stress in atherosclerosis.

Compliance with Ethics Guidelines

Conflict of Interest Ajoe John Kattoor, Naga Venkata K Pothineni, Deepak Palagiri, and Jawahar L. Mehta declare no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of Major Importance

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