Review

Atherosclerosis and oxidative stress

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Summary. This review focuses on the morphological features of atherosclerosis and the involvement of oxidative stress in the initiation and progression of this disease. There is now consensus that atherosclerosis represents a state of heightened oxidative stress characterized by lipid and protein in the vascular wall. Reactive oxygen species (ROS) are key mediators of signaling pathways that underlie vascular inflammation in atherogenesis, starting from the initiation of fatty streak development, through lesion progression, to ultimate plaque rupture. Plaque rupture and thrombosis result in the acute clinical complications of myocardial infarction and stroke. Many data support the notion that ROS released from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, myeloperoxidase (MPO), xanthine oxidase (XO), lipoxygenase (LO), nitric oxide synthase (NOS) and enhanced ROS production from dysfunctional mitochondrial respiratory chain, indeed, have a causatory role in atherosclerosis and other vascular diseases. Moreover, oxidative modifications in the arterial wall can contribute to the arteriosclerosis when the balance between oxidants and antioxidants shifts in favour of the former. Therefore, it is important to consider sources of oxidants in the context of available antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase and transferases thiol-disulfide oxidoreductases and peroxiredoxins. Here, we review also the mechanisms in which they are involved in order to accelerate the pace of the discovery and facilitate development of novel therapeutic approaches.

Key words: Reactive oxygen species, Antioxidant enzymes, Oxidative stress, Vascular damage

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Introduction

Marchand introduced the term "atherosclerosis", describing the association of fatty degeneration and vessel stiffening (Aschoff, 1933; Crowther, 2005). This process affects medium and large-sized arteries and is characterized by patchy intramural thickening of the subintima that encroaches on the arterial lumen. Each vascular bed may be affected by this process; the etiology, treatment and clinical impact of atherosclerosis vary from one vascular bed to another (Faxon et al., 2004). The earliest visible lesions of atherosclerosis are the fatty streak, which is due to an accumulation of lipid-laden foam cells in the intimal layer of the artery. With time, the fatty streak evolves into a fibrous plaque, the hallmark of established atherosclerosis. Ultimately the lesion may evolve to contain large amounts of lipid; if it becomes unstable, denudation of overlying endothelium or plaque, or plaque rupture, may result in thrombotic occlusion of the overlying artery.

Atherosclerosis, in fact, is a progressive disease characterized by the accumulation of cholesterol deposits in macrophages (foam cells) in large and medium arteries. This deposition leads to a proliferation of certain cell types within the arterial wall, which gradually impinge on the vessel lumen and impede blood flow. These early lesions, called "fatty streak lesions", can usually be found in the aorta in the first decade of life, in the coronary arteries in the second decade, and in the cerebral arteries in the third or fourth decades. Fatty streaks are not clinically significant, but they are the precursors of more advanced lesions characterized by the accumulation of lipid-rich necrotic debris and smooth muscle cells (SMCs) (Lusis, 2000). Atherosclerosis manifests itself histological as arterial lesions known as plaques, which have been extensively characterized. Plaques contain a central lipid core that is most often hypocellular and may even include crystals of cholesterol that have formed in the aftermath of necrotic

foam cells. The lipid core is separated from the arterial lumen by a fibrous cape and myeloproliferative tissue that consists of extracellular matrix and smooth muscle cells (Stocker and Keaney, 2004). Plaques can become increasingly complex, with calcification, ulceration at the luminar surface, and hemorrhages from small vessels that grow into the lesion from the media of the blood vessel wall. Although advanced lesions can grow sufficiently large to block blood flow, the most important clinical complication is an acute occlusion due to the formation of a thrombus or blood clot, resulting in myocardial infarction or stroke. Age, gender, obesity, cigarette smoking, hypertension, diabetes mellitus and dyslipidemias are known atherogenic risk factors that promote the impairment of endothelial function, smooth muscle function and vessel wall metabolism. These risk factors are associated with an increased production of free radicals that are called ROS (Antoniades et al., 2003). ROS are metabolites of oxygen that, through their high reactivity, are prone to participation in oxidation-reduction reactions. An increasing number of studies have demonstrated that oxidative stress (dysregulation of the cellular redox state) plays a pivotal role in the pathogenesis of atherosclerosis, especially vascular endothelial dysfunction. Reactive oxygen species have detrimental effects on vascular function through several mechanisms. First, ROS, especially hydroxyl radicals, directly injure cell membranes and nuclei. Second, by interacting with endogenous vasoactive mediators formed in endothelial cells, ROS modulate vasomotion and the atherogenic process. Third, ROS peroxidize lipid components, leading to the formation of oxidized lipoproteins (LDL), one of the key mediators of atherosclerosis. Whereas native LDL does not cause cholesterol ester accumulation in macrophages, LDL modified by oxidation does. Oxidized LDL has also been implicated in other mechanisms potentially involved in the development of atherosclerosis, such as cytotoxic or chemotactic actions on monocytes and the inhibition of macrophage motility (Inoue and Node, 2006). Up-regulation of adhesion molecules, such as intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), platelet-endothelial cell adhesion molecule-1 (PECAM-1), integrins, and selectins (L, E, and P) will lead to the attachment and buildup of immune cells (monocytes, macrophages, platelets, and T lymphocytes) on the endothelial wall. This activation of the endothelium leads to the release of pro-inflammatory cytokines, surface receptors, and proteinase enzymes including, but not limited to, interleukins, interferons, monocyte chemoattractant protein-1 (MCP-1), monocyte chemoattractant protein-4 (MCP-4) and cyclooxygenase-2 (COX-2). The endothelium becomes more permeable to lipid particles and immune cells, which leads to a situation where macrophages engulf oxidized low-density LDL particles and other modified lipoproteins, thus becoming foam cells. Foam cells in turn lead to an increase in inflammatory cytokine production (Fig. 1). This proliferating immune response activates the vascular smooth muscle cell (VSMC) layer to proliferate and migrate as well as leading to local vasoconstriction, collagen and matrix deposition, fibrous cap production, and immune cell and platelet recruitment and activation (Hennig et al., 2001). Here, we considered the morphological features of atherosclerosis and the
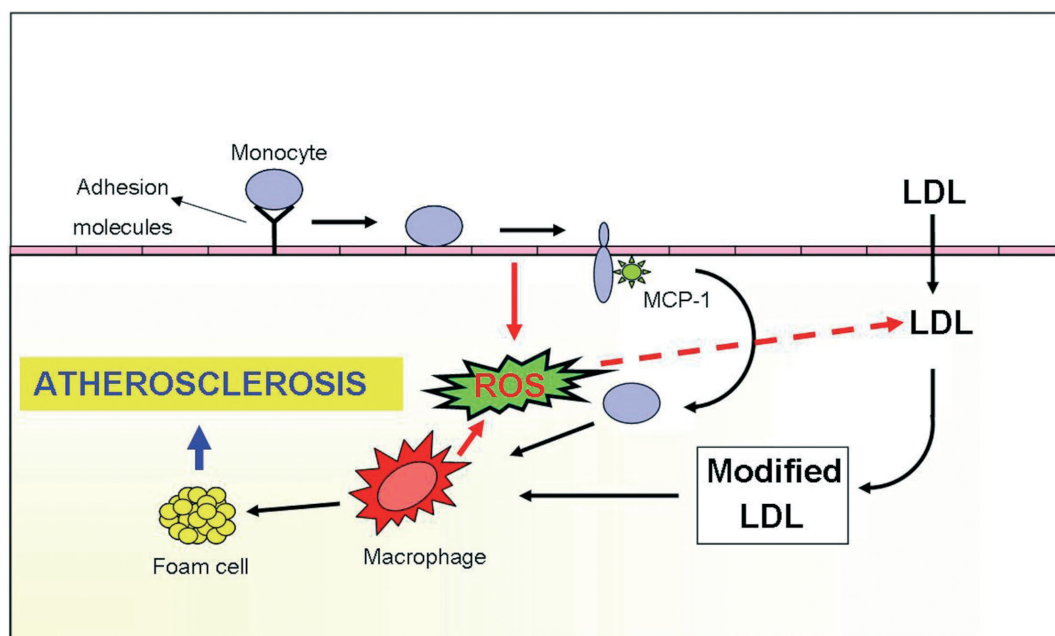
Fig. 1. Oxidative stress in atherosclerosis. An increase of free radical production is associated with atherosclerosis. These radicals provoke an oxidative modification from low-density lipoprotein (LDL) to oxidized low-density lipoprotein (ox-LDL). Circulating monocytes migration to the subendothelial space is stimulated by ox-LDL and also causes endothelial cell injury. The modified LDL is taken up by macrophages which become foam cells, leading to the formation of atherosclerotic plaque.

involvement of oxidative stress in the initiation and progression of this disease. In particular, we focused on pro-oxidant factors and on the main endogenous protection mechanisms, such as antioxidant enzymes.

Morphological features of atherosclerosis

Atherosclerosis manifests itself histologically as arterial lesions known as plaques, which have been extensively characterised into six major types of lesions reflecting the early, developing and mature stages of the disease (Stary et al., 1995) (Fig. 2). In the arterial tree sites prone to the disease, adaptive thickening of the intima is among the earliest histological changes. As macrophages accumulate lipid, type II lesions form as nodular areas of lipid deposition that are also known as "fatty streaks", and these represent lipid-filled macrophages (i.e. foam cells). Continued foam cell formation and macrophage necrosis can produce type III lesions containing small extracellular pools of lipid. Type IV lesions are defined by a relatively thin tissue separation of the lipid core from the arterial lumen, whereas type V lesions exhibit fibrous thickening of this structure, also known as the lesion "cap". Recent data showed that mature type VI lesions exhibit a complicated architecture with plaque formation and progression, culminating in their rupture.

Plaque morphology

The light microscopical appearance is visible in Fig. 3, showing histopathologic changes observed in apolipoproteins E-deficient (Apo-E°) mice. These mice are a useful tool for experimental studies, since they develop hypercholesterolemia and accelerated atherosclerosis, and their atherosclerotic lesion composition is similar to human atherosclerotic lesions (Coleman et al., 2006; Rodella et al., 2007). The plaques are characterised by a growing mass of extracellular lipids protruding into the lumen of the vessels; lipid droplets are associated with the intimal cells of the vessels, especially in lipid-rich macrophages ("foam cells"). These early developing lesions typically developed acellular regions, characterized by necrotic lipid cores. In several cases, the raised lesions are covered by a collagenous cap with myeloproliferative tissue, which consists of extracellular matrix and SMCs. Substantial collagen deposition is associated with the VSMCs, many of which also show cytoplamic lipid inclusions. Elastin laminae are fragmented, discontinuous and commonly display reduced thickness (Rodella et al., 2007).
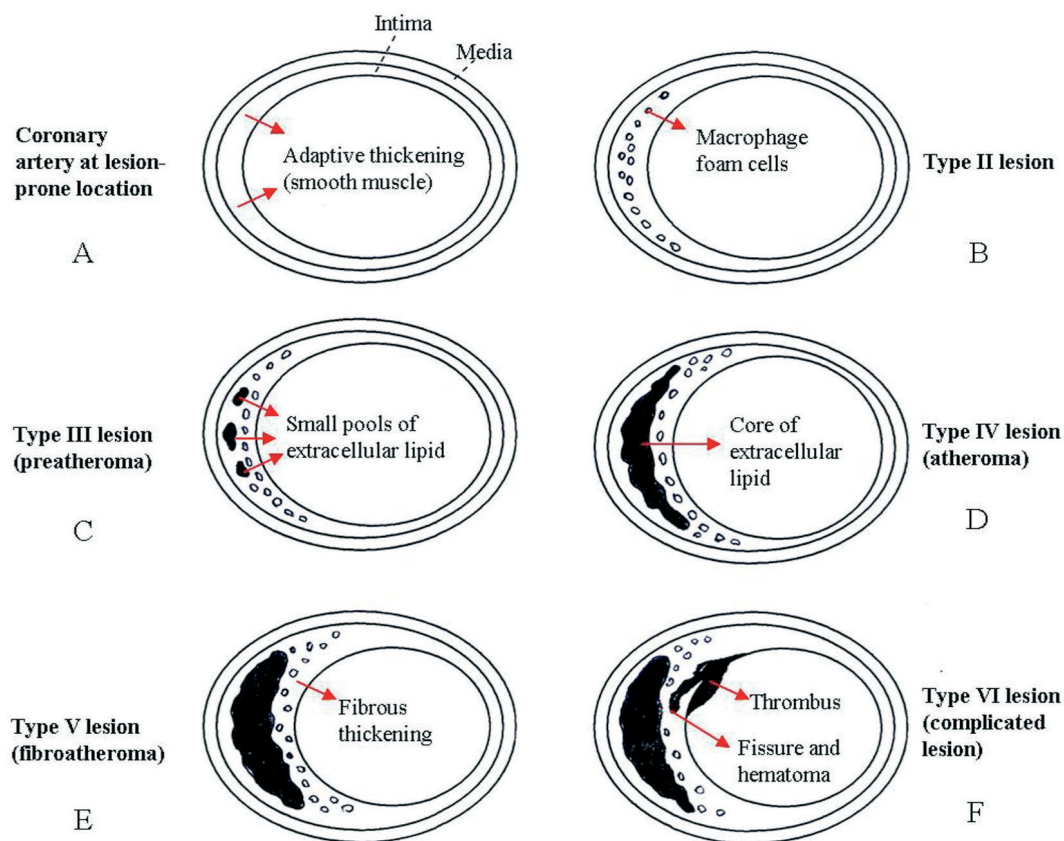
Fig. 2. Early, developing and mature stages of atherosclerosis. **A** shows earliest stage. **B,C,D and E** show developing stages of the disease. **F** shows an advanced stage that culminates in plaque rupture and thrombosis.

Plaque rupture

Over the last twenty years, the term "plaque rupture" consists of a number of events responsible for atherosclerosis progression and considerable efforts have been directed at understanding the composition and the vulnerability of plaques, rather than the severity of the stenosis. In fact, mature atherosclerotic plaques have been subdivided in stable and vulnerable plaques. Vulnerable plaques generally have thin fibrous caps and increased numbers of inflammatory cells (Lusis, 2000). Maintenance of the fibrous cap reflects matrix production and degradation, and products of inflammatory cells are likely to influence both processes. For example, T cells produce interferon-γ (IFN-γ), which inhibits the production of matrix by SMCs, and macrophages produce various proteases that degrade extracellular matrix, including interstitial collagenase, gelatinases and stromolysin. Rupture frequently occurs at the lesion edges, which are rich in foam cells, suggesting that factors, contributing to inflammation, may also influence thrombosis. In this regard, it is important to note that the incidence of myocardial infarction and stroke increases during acute infections.

Moreover, the stability of atherosclerotic lesions may also be influenced by calcification and neovascularization, common features of advanced lesions. Intimal calcification is an active process in which pericyte-like cells secrete a matrix scaffold, which subsequently becomes calcified, akin to bone formation. The process is regulated by oxysterols and cytokines (Moultan and Folkman, 1999). The thrombogenicity of the lesion core is likely to depend on the presence of tissue factor, a key protein in the initiation of the coagulation cascade. The production of tissue factors by endothelial cells (ECs) and macrophages is enhanced by oxidized LDL, infection or the ligation of CD40 on ECs to CD40L on inflammatory cells. The expression of other molecules mediating thrombosis, such as plasminogen activator, may also be important (Geller et al., 2000).

Oxidative stress

Oxidative stress: a definition

The notion of "oxidative stress" in biological systems goes back to the early period of research on oxygen activation with an initial focus on oxygen toxicity and X-irradiation (Stocker and Keaney, 2004). Much of the relevant literature was reviewed in 1979 by Chance et al. (1979) in an article on hydroperoxyde metabolism in mammalian organs. In the following paper, the concept of oxidative stress was developed primarily by Sies (1985), with synonymous terms such as "oxidant stress" and "pro-oxidant stress", or the related term "reductive stress" receiving comparatively less emphasis. Sies described oxidative stress as a "disturbance in the pro-oxidant/oxidant balance in favour of the former" (Sies, 1985). This original denotation has been modified since to the more refined definition of "imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to damage" (Sies, 1991). This more careful definition accounts for more important operational considerations. For example, an oxidative challenge or a loss of antioxidants alone does not constitute oxidative stress. However, if increased formation of oxidant(s) is accompanied by a loss of antioxidant(s) and/or accumulation of oxidized forms of the antioxidant(s), oxidative stress is approached. The refined definition also conceptually distinguishes oxidative stress from
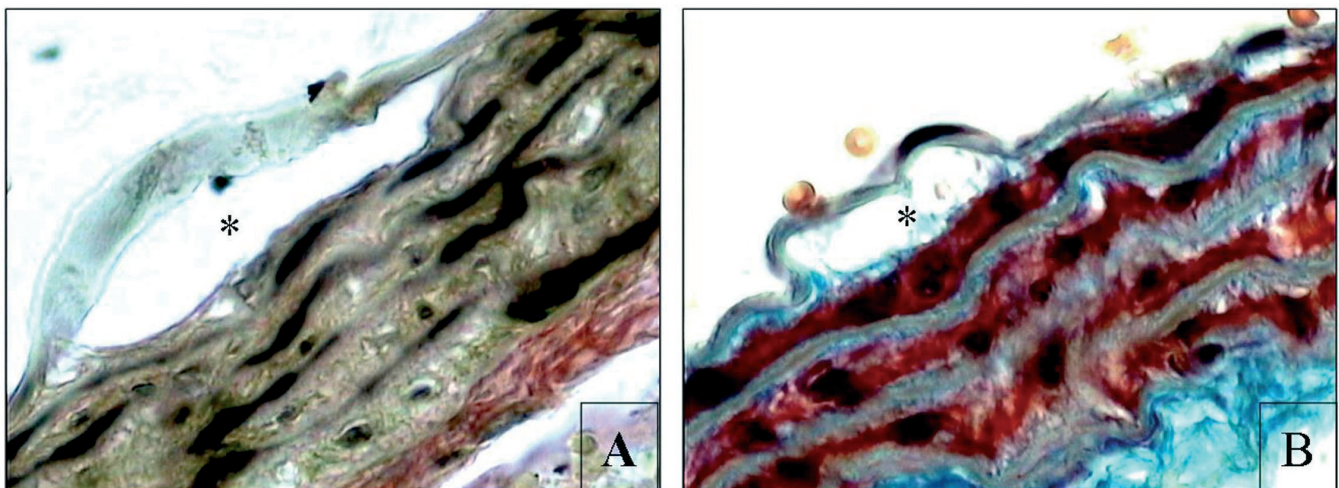
Fig. 3. Histology of an atherosclerotic palque in aorta stained with verhoeff's elastin stain (**A**) and Masson's trichrome stain (**B**). The asterisk indicates the plaque. x 1,000

oxidative damage. Thus even an oxidative assault that is accompanied by a loss of antioxidants may not necessarily result in oxidative damage. For example, biological systems are characterized by adaptive responses that may compensate and perhaps even overcompensate the oxidative stress, which may manifest itself as a situation of increased redox environment. The refined definition of oxidative stress and its underlying redox chemistry, involving reduction-oxidation reactions, implies that any form of "tipping the balance" causes an "imbalance". This has led to the concept of reductive stress to describe a situation where the balance is altered. Healthy vascular cells metabolize oxygen and generate in favour of reductans. Reductive stress can be intimately linked to oxidative stress. For example, an overproduction of reducing equivalents, such as NAD(P)H, may result in increased redox cycling of substances that can undergo repetitive rounds of oxidation/reduction, ultimately leading to the increased generation of superoxide anion radical ($O2-$) and secondary oxidants. This has been implicated in the formation of ROS by hypoxia-like metabolic imbalances (Tilton et al., 1997).

With the increased appreciation of interplay between ROS and reactive nitrogen species (RNS), including their responses in cells, the term "nitrosative stress" has also been introduced. Nitrosative stress, defined as an increase in S-nitrosated compounds, associated with a decrease in intracellular thiols, may be associated with a number of biological responses, some of which are of particular interest to vascular physiology and pathophysiology.

Because of the apparent importance of ROS in vascular disease, there is substantial interest in the enzymatic sources that contribute to production of free radicals in vascular tissues. Several enzyme systems seem to be important in this process, including XO, the NADPH- oxidase, MPO, LPO, mitochondrial sources and NOS (Harrison et al., 2003).

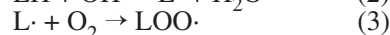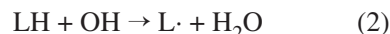*Oxidants and markers of oxidants events*

A free radical can be defined as any species capable of independent existence that contains one or more unpaired electrons. In biological systems, a variety of radicals can be generated (Table 1) with their reactivity depending on their nature and the molecule(s) encountered. If two radicals meet, they can join their unpaired electrons to form a covalent bond in reactions that are often kinetically fast and that lead to nonradical products. An example relevant to the vessel wall is the very fast reaction of O_2^- with NO to form peroxynitrite ($ONOO^-$) (reaction 1)

$$O_2^{-}\cdot + \cdot NO \rightarrow ONOO^- \qquad (1)$$

Alternatively, a radical may add to a nonradical molecule or abstract a hydrogen atom from C-H, O-H, or S-H bond of nonradical molecules. These types of

radical reaction are common in biological systems where most molecules are nonradical species. The molecules potentially affected include low-molecular-weight compounds like antioxidants and cofactors of enzymes, lipids, proteins, nucleic acids and sugars. In this case, a new radical is generated and this can set up a chain reaction.

A typical example of such a chain reaction is the process of lipid peroxidation that may be initiated by, for example, a hydroxyl radical ($\cdot \dot{O}H$) abstracting a hydrogen atom from a fatty acid side chain (LH) containing carbon atoms with bisallylic hydrogens (reaction 2). The resulting carbon-centered radical ($LOO\cdot$) (reaction 3) which itself can propagate the chain by reacting with a neigh-boring lipid molecule to generate another $L\cdot$ and lipid hydroperoxide (LOOH) (reaction 4). In this fashion, many molecules of LOOH may be generated for each initiating radical.

$$\begin{array}{ll} LH + OH \rightarrow L\cdot + H_2O & (2) \\ L\cdot + O_2 \rightarrow LOO\cdot & (3) \\ LOO\cdot + LH \rightarrow L\cdot + LOOH & (4) \end{array}$$

Whereas the highly reactive $\cdot OH$ abstracts H atoms almost with discrimination, less reactive radicals, such as $LOO\cdot$ preferentially abstract H atoms from molecules with weaker bonds, such as the chromanol O-H bond contained in α-tocopherol (α-TOH). The α-tocopheroxil radical (α-TO$\cdot$) is produced and, for $LOO\cdot$, a molecule of LOOH (reaction 5):

$$LOO\cdot + \alpha\text{-TOH} \rightarrow LOOH + \alpha\text{-TO}\cdot$$
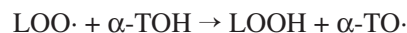
A radical may be an oxidizing agent, accepting a single electron from nonradical, or a reducing agent, donating a single electron to a nonradical. Buettner (1993) has compiled a useful list of biologically relevant standard reduction potentials that predict the directions of reactions. Accordingly, the ascorbate/ascorbyl radical system is, for example, capable of reducing the α-TO$\cdot$ / H^+ α -TOH system (reaction 6) that has a more positive standard reduction potential.

$$H^+ + \text{ascorbate}^- + \alpha\text{-TO}\cdot \rightarrow \alpha\text{-TOH}\cdot + \text{ascorbyl radical}$$
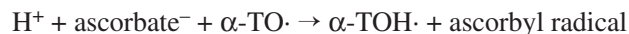
Oxidants

In addition to radicals, several nonradical oxidants are important when considering oxidative modifications in the vessel wall (Table 1). Arguably, the most abundant of these is hydrogen peroxide (H_2O_2) derived from the action of oxidases such as glucose oxidase on O_2 or from the dismutation of O_2^- (reaction 7):

$$2O_2^{-}\cdot + 2H^+ \rightarrow H_2O_2 + O_2$$
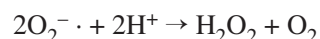
As in the case for radicals, the reactivity of the different radical species varies. Hydrogen peroxide is generally a weak oxidant, although it can directly

oxidize thiol (-SH) groups, for example, at the active site of enzymes like glyceraldehyde-3-phosphate dehydrogenase. Nonradical oxidants like ONOOHH and hypochlorous acid (HOCl) appear to react preferentially with proteins rather than lipids. A major biological target for $ONOO^-$ is carbon dioxide (CO_2). The reaction of $ONOO^-$ with CO_2 is complex and produces metastable species that promote nitration, nitrosation and oxidation reaction.

Sources of oxidants and markers of oxidative enzymes

Several lines of evidence implicate ROS and RNS in atherogenesis. The advent of gene targeting to predictably modify genes has generated valuable animal models and allowed a genetic approach to gain information on how oxidants affect disease. The development of sensitive analytical methods also enables researchers to obtain direct chemical evidence for specific reaction pathways that promote oxidative events and lesion formation in vivo, and to relate the accumulation of specific oxidized lipid and protein to different stages of atherosclerosis. Understanding the processes that lead to the different oxidative events is important in developing strategies to effectively inhibit such processes and hence, potentially, atherosclerosis. Within the vessel wall, the different oxidants can originate principally from cellular and extracellular sources, and from enzymatic and nonenzymatic paths that are reviewed briefly in the following section.

XO

XO is an iron sulfur molybdenum flavoprotein with multiple functions and present in high concentrations in ECs of capillary and sinusoids. It exists in two forms, xanthine dehydrogenase and XO, of which the former is predominant. It generates O_2^- by catalyzing hypoxanthine and xanthine to uric acid. Under pathophysiologic conditions, this is another major source of vascular oxidative stress (Dröge, 2002). XO exists in plasma and endothelial cells but not in smooth muscle cells (SMCs) (Harrison et al., 2003).

The role for XO in atherosclerosis is further corroborated by the following observations (Spiekermann et al., 2003):
- In the coronary arteries of patients with coronary artery disease (CAD), electron spin resonance studies show significant activation of XO.
- In these same patients, endothelial XO is inversely proportional and positively related to the effect of vitamin C on endothelium-dependent vasodilatation.
- In asymptomatic young individuals with familial hypercolesterolemia, the increase of vascular XO activity is an early event.

NAD(P)H oxidases

It has long been known that phagocytes, including neutrophils, monocytes and macrophages contain a plasma membrane-bound, multicomponent oxidase that utilizes electrons derived from NADPH to reduce molecular oxygen to O_2^-·. While O_2^-· is principally a reducing agent, it can give rise to secondary products that include strong oxidants.

The NADPH oxidases have emerged as very important sources of ROS in vascular cells. An important aspect of this enzyme system is that it is regulated by a variety of pathophysiologic stimuli relevant to atherosclerosis (Harrison et al., 2003). Angiotensin II, platelet derived growth factor (PDGF), and tumor necrosis factor α (TNF-α) may increase ROS in the atherosclerotic lesion by stimulating the local vascular myocytes to produce ROS. Subsequently, ROS may contribute to LDL oxidation, local MCP-1 production, upregulation of adhesion molecules and macrophage recruitment, endothelial dysfunction, and extracellular matrix remodeling through collagen degradation and eventually plaque rupture (Griendling et al., 2000).

MPO

It is a heme-containing enzyme that generates HOCl

Table 1. Free redicals generation and cellular antioxidants in biological systems.

Examples of free radicals in biological systems	Examples of nonradical oxidants of potential relevance in oxidative stress	Cellular antioxidants
Carbon centered radical	Hydrogen peroxide	Cu, Zn-SOD
Superoxide anion	Hypochlorite	Mn-SOD
Hydroperoxyl radical	Hypochlorus acid	Catalase
Peroxyl radical	Ozone	Glutathione peroxidase
Alkoxyl radical	Singlet oxygen	Glutathione reductase
Hydroxyl radical	Oxoperoxonitrate or peroxynitrite	Thiol-disulfide oxidoreductases
Nitric oxide	Peroxinitrous acid	Peroxiredoxin
Nitrogen dioxide	Alkylperoxynitrites	Methionine sulfoxide reductases
Thiyl radical	Nitryl chloride	Heme oxygenase
Perthiyl radical	Nitronium ion	Ferritin
Transition-metal ions	Nitrosothiols	GSH

and chlorinated biomolecules, which are therefore considered specific markers of oxidation reactions catalyzed by the enzyme. There is a growing body of evidence suggesting that MPO might play a role in different mechanisms that damage the human artery wall. MPO is an enzyme that is stored within the azurophile granules of neutrophils and monocytes, which generate modified and (or) oxidized (lipo) proteins via the production of HOCl from H_2O_2 and chloride. MPO, HOCl-modified proteins, and HOCl-modified LDL (HOCl-LDL) are present in human atherosclerotic lesions, where they are located both in vascular cells and extracellular spaces (Daugherty et al., 1994). Recent clinical studies have shown that a MPO deficiency, or a low level of blood MPO, had beneficial effects against cardiovascular damage in patients presenting with this deficiency (Kutter et al., 2000; Zhang et al., 2001). Two other reports demonstrated that the MPO serum level is a predictor of risk in patients with acute coronary syndromes or chest pain (Baldus et al., 2003; Brennan et al., 2003). The oxidation of LDL by MPO mainly leads to modifications of apo-lipoproteins with the formation of chlorotyrosine, dityrosine, and nitrotyrosine (Heinecke, 2003), the lipidic moiety attack being more dependent on pH and the presence of preformed hydroperoxide (Spickett et al., 2000). Shishehbor et al. (2003) reported such changes in plasma proteins from hypercholesterolemic subjects with no known coronary artery disease. In addition, we observed that MPO can use the H2O2 produced by endothelial cells to oxidize LDL in vitro at their surface (Zouaoui Boudjeltia et al., 2004).

LO

Another important ROS-generating system is represented by the LO; they are iron-containing dioxygenases that catalyze the sterospecific insertion of molecular oxygen into polyunsaturated fatty acids to give rise to a complex family of biologically active lipids, including prostaglandins, thromboxanes and leukotrienes. Prostaglandins and thromboxanes comprise metabolites from arachidonate and similar fatty acids. The LO are non-heme iron-containing dioxygenases that catalyze stereospecific insertion of molecular oxygen into polyunsaturated fatty acids, yielding a family of biologically active lipids, including prostaglandins, tromboxanes and leucotrienes that contribute to inflammatory reactions and can increase vascular permeability. LO require low levels of "seeding peroxides" to oxidize inactive Fe^{++} to active Fe^{+++} enzyme, and are probably affected by the "peroxide tone" of cells (Stocker and Keaney, 2005). Leucocyte-type 12/15-LO and its products have been implicated in atherogenesis. The first direct proof in vivo of a role for the lipoxygenase pathway was provided by Cyrus and colleagues (1999) in Apo E null mice that were made genetically deficient in 12/15-lipoxygenase and that were found to have significantly less atherosclerosis as a result. Deletion of the 12/15-lipoxygenase gene in LDL receptor null mice and in macrophages of mice that were both LDL receptor null and also deficient in the apoB-editing catalytic polypeptide-1 enzyme resulted in decreased atherogenesis in both mouse models. Conversely, overexpression of the 12/15-lipoxygenase gene in the endothelium of LDL receptor null mice accelerated atherosclerosis (Navab et al., 2004).

Mitochondrial sources

Mitochondria provide energy (adenosine triphosphate, ATP) to the cell through oxidative phosphorylation. Oxidative phosphorylation is the process by which ATP is formed as electrons are transferred from NADH or $FADH_2$ to molecular oxygen. This occurs through a series of electron transport carriers localized in the inner mitochondrial membrane (Singh and Jialal, 2006). It is becoming increasingly clear that under pathological conditions, mitochondrial oxidative phosphorylation can become uncoupled and results in the generation of $O_2^{\bullet-}$.

The paradigm that mitochondrial ROS are key determinants of myocardial dysfunction has been addressed by a number of investigators in both experimental animal systems and humans (Madamanchi et al., 2005). Mitochondrial ROS are clearly associated with early atherosclerotic lesion development, and emerging evidence indicates that many cardiovascular syndromes are associated with some evidence for mitochondrial dysfunction, although a causal role has yet to be established in vivo (Singh and Jialal, 2006).

NOS

NOS are a family of enzymes that catalyze the oxidation of L-arginine to L-citrulline and the potent vasodilator $NO^{\bullet}$. In the context of the vasculature and atherosclerosis, endothelial NOS (eNOS) and inducible NOS (iNOS) are most relevant. The activity of eNOS, as opposed to iNOS and strong evidence, suggests that eNOS plays an important role in protection of the vessel wall from atherosclerosis, yet the role of iNOS in vascular pathology is variable and poorly defined in atherosclerosis (Singh and Jialal, 2006). Under normal conditions, eNOS requires tetrahydrobiopterin (BH_4), bound near this heme group, to transfer electrons to a guanidino nitrogen of L-arginine to form nitric oxide. In the absence of either L-arginine or BH_4, eNOS can produce $O_2^{\bullet-}$ and H_2O_2. This phenomenon has been referred to as NOS uncoupling. Uncoupling of eNOS in the endothelium may lead to oxidative stress and endothelial dysfunction via at least 3 mechanisms. First, the enzymatic production of $NO^{\bullet}$ is diminished, allowing the radicals that it normally might react with to attack other cellular targets. Second, the enzyme begins to produce O_2^-, contributing to oxidative stress. Finally, it is likely that eNOS can become partially uncoupled, such that both $O_2^{-\bullet}$ and $NO^{\bullet}$ are produced

simultaneously. Under this circumstance, eNOS may become a peroxynitrite generator, leading to a dramatic increase in oxidative stress (Cai and Harrison, 2000).

The redox equilibrium between NO and oxidative stress has a profound impact on expression of genes in the vessel wall, related to progression and vulnerability of atherosclerotic lesions as well as on parameters of inflammation and cell apoptosis. Oxidation is essential for life; however, it is deleterious for the vasculature if oxidative processes are out of control, as seen in endothelial dysfunction and subsequent atherosclerosis. Experimental findings indicate that in atherosclerosis the activity of endothelial NO synthase is reduced. However, total NO production might be enhanced, since NO is not only produced by endothelial NO synthase, but also through neuronal NO synthase, and more importantly, by inducible NO synthase in macrophages and other cell types in the atherosclerotic plaque (Schächinger and Zeiher, 2002).

Antioxidant enzyme systems

Oxidative modifications within the arterial wall that may initiate and/or contribute to atherogenesis likely occur when the balance between oxidants and antioxidants shifts in favour of the former. Therefore, it is important to consider sources of oxidants in the context of available antioxidants. Many substances prevent, or significantly delay, the oxidation of other substrates. However, antioxidant is defined as a substance that it protects. With regard to atherosclerosis, vascular antioxidants need to protect against 2e-oxidants, both within and outside cells. In the context of the oxidative modification hypothesis, antioxidant protection of LDL in the extracellular space deserves focus, as oxidized LDL has many potential proatherogenic activities and the cellular accumulation of oxidized LDL is considered a hallmark of atherosclerosis (Steinberg et al., 1989). In addition, cellular antioxidants are probably important in the context of the presence of heightened oxidative stress within the vessel wall and the known effect of oxidative events on key cellular activities such as ·NO-related bioactivities. The following review briefly describes the antioxidant defenses in arterial wall cells and lipoproteins that counteract oxidative modifications.

Enzymatic antioxidants

The classic antioxidant enzymes are largely cell-associated proteins whose function is to maintain a reducing tone within cells (Table 1); they may also be involved in the maintenance of extracellular antioxidants. Enzymatic antioxidants principally include SOD, CAT, GPx, glutathione reductase, transferases thiol-disulfide oxidoreductases, and peroxiredoxins. Many of these enzymatic antioxidants are present in normal arteries, most likely within vascular wall cells as extracellular fluid is largely devoid of enzymatic antioxidants (Hamilton et al., 2004).

SOD

SODs are a major cellular defense system against superoxide in all vascular cells. These enzymes contain redox metal in the catalytic center, and dismutase superoxide radicals to hydrogen peroxide and oxygen. Three different isoforms of SOD have been identified: the mitochondrial manganese-containing SOD (MnSOD, SOD2), the cytosolic copper/zinc-containing SOD (CuZnSOD, SOD1), and the extracellular SOD (ecSOD, SOD3) (Hamilton et al., 2004; Wassmann et al., 2004).

CAT

CAT is an intracellular antioxidant enzyme that is mainly located in cellular peroxisomes and to some extent, in the cytosol, which catalyzes the reaction of hydrogen peroxide to water and molecular oxygen in a 2-step reaction. CAT is very effective in high-level oxidative stress and protects cells from hydrogen peroxide produced within the cell. The enzyme is especially important in the case of limited glutathione content or reduced glutathione peroxidase activity and plays a significant role in the development of tolerance to oxidative stress in the adaptive response of cells (Wassmann et al., 2004).

GPx

Reduced glutathione plays a major role in the regulation of the intracellular redox state of vascular cells by providing reducing equivalents for many biochemical pathways. GPx is a selenium-containing antioxidant enzyme that effectively reduces hydrogen peroxide and lipid peroxides to water and lipid alcohols, respectively, and in turn oxidizes glutathione to glutathione disulfide. The GPx/glutathione system is thought to be a major defense in low-level oxidative stress (Wassmann et al., 2004).

Thiol-disulfide oxidoreductases

The protein disulfide isomerases are a group of enzymes important in the correct folding of proteins during their synthesis, in the control of the redox state of existing exofacial protein thiols or reactive disulfide bonds, and in the "ordered movement" of ·NO across the plasma membrane (Stocker and Keaney, 2004). They are produced, and at least some of them may be secreted, by various cells, raising the possibility that they also contribute to the antioxidant defense in extracellular fluid, although it is unknown whether they can mediate the reduction of lipoprotein lipid hydroperoxides.

Peroxiredoxin

Peroxiredoxins are a family of antioxidant enzymes

that comprises several members located in the cytosol, mitochondria, peroxisomes and plasma membrane of cells as well as in plasma. The enzymes generally exhibit peroxidase activity and appear to serve a variety of functions associated with different biological processes, such as cell proliferation, differentiation and gene expression (Fujii and Ikeda, 2002).

Conclusion

The growing evidence from data of experimental and clinical studies suggests that oxidative stress is implicated in atherosclerotic diseases. However, a better understanding of ROS production mechanisms and signaling pathways in vascular pathophysiology is a prerequisite for effective pharmacological interventions for vascular disease. Moreover, the knowledge of the complexity of cellular redox reactions and the utilization of natural antioxidants targeted to specific subcellular organelles will likely be avenues for future research toward the broader use of pharmacological therapies in the treatment and prevention of atherosclerosis.

References

Antoniades C., Tousoulis D., Tentolouris C., Toutouzas P. and Stefanadis C. (2003). Oxidative stress, antioxidant vitamins, and atherosclerosis. From basic research to clinical practice. Herz 28, 628-638.

Aschoff L. (1933). Introduction. In: Arteriosclerosis: A survey of problem. Cowdry E.V. (ed). Macmillan. New York.

Baldus S., Heeschen C., Meinertz T., Zeiher A.M., Eiserich J.P., Munzel T., Simoons M.L. and Hamm C.W. (2003). Myeloperoxidase serum levels predict risk in patients with acute coronary syndromes. Circulation 108, 1440-1445.

Brennan M.L., Penn M.S., Van Lente F., Nambi V., Shishehbor M.H., Aviles R.J., Goormastic M., Pepoy M.L., McErlean E.S., Topol E.J., Nissen S.E. and Hazen S.L. (2003). Prognostic value of myeloperoxidase in patients with chest pain. N. Engl. J. Med. 349, 1595-1604.

Buettner G.R. (1993). The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. Arch. Biochem. Biophys. 300, 535-543.

Cai H. and Harrison D.G. (2000). Endothelial dysfunction in cardiovascular diseases. The role of oxidant stress. Circ. Res. 87, 840-844.

Chance B., Sies H. and Boveris A. (1979). Hydroperoxide metabolism in mammalian organs. Physiol. Rev. 59, 527-605.

Coleman R., Hayek T., Keidar S. and Aviram M. (2006). A mouse model for human atherosclerosis: long-term histopathological study of lesion development in the aortic arch of apolipoprotein E-deficient (E0) mice. Acta Histochem. 108, 415-424.

Crowther M.A. (2005). Pathogenesis of atherosclerosis. Hematology. Am. Soc. Hematol. Educ. Program. 436-441.

Cyrus T., Witztum J.L., Rader D.J., Tangirala R., Fazio S., Linton M.F.

and Funk C.D. (1999). Disruption of the 12/15-lipoxygenase gene diminishes atherosclerosis in apo E-deficient mice. J. Clin. Invest. 103, 1597-1604.

Daugherty A., Dunn J.L., Rateri D.L. and Heinecke J.W. (1994). Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. J. Clin. Invest. 94, 437-444.

Droge W. (2002). Free radicals in the physiological control of cell function. Physiol. Rev. 82, 47-95.

Faxon D.P., Fuster V., Libby P., Beckman J.A., Hiatt W.R., Thompson R.W., Topper J.N., Annex B.H., Rundback J.H., Fabunmi R.P., Robertson R.M. and Loscalzo J. (2004). Atherosclerotic Vascular Disease Conference: Writing Group III: pathophysiology. Circulation 109, 2617-2625.

Fujii J. and Ikeda Y. (2002). Advances in our understanding of peroxiredoxin, a multifunctional, mammalian redox protein. Redox Rep. 7, 123-130.

Geller D.S., Farhi A., Pinkerton N., Fradley M., Moritz M., Spitzer A., Meinke G., Tsai FT., Sigler P.B. and Lifton R.P. (2000). Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy. Science 289, 119-123.

Griendling K.K., Sorescu D. and Ushio-Fukai M. (2000). NAD(P)H oxidase: role in cardiovascular biology and disease. Circ. Res. 86, 494-501.

Hamilton C.A., Miller W.H., Al-Benna S., Brosnan M.J., Drummond R.D., McBride M.W. and Dominiczak A.F. (2004). Strategies to reduce oxidative stress in cardiovascular disease. Clin. Sci. (Lond). 106, 219-234.

Harrison D., Griendling K.K., Landmesser U., Hornig B. and Drexler H. (2003). Role of oxidative stress in atherosclerosis. Am. J. Cardiol. 91, 7A-11A.

Heinecke J.W. (2003). Oxidative stress: new approaches to diagnosis and prognosis in atherosclerosis. Am. J. Cardiol. 91, 12A-16A.

Hennig B., Toborek M. and McClain C.J. (2001). High-energy diets, fatty acids and endothelial cell function: implications for atherosclerosis. J. Am. Coll. Nutr. 20, 97-105.

Inoue T. and Node K. (2006). Vascular failure: A new clinical entity for vascular disease. J. Hypertens. 24, 2121-2130.

Kutter D., Devaquet P., Vanderstocken G., Paulus J.M., Marchal V. and Gothot A. (2000). Consequences of total and subtotal myeloperoxidase deficiency: risk or benefit ? Acta Haematol. 104, 10-15.

Lusis A.J. (2000). Atherosclerosis. Nature 407, 233-241.

Madamanchi N.R., Hakim Z.S. and Runge M.S. (2005). Oxidative stress in atherogenesis and arterial thrombosis: the disconnect between cellular studies and clinical outcomes. J. Thromb. Haemost. 3, 254-267.

Moultan K.S. and Folkman J. (1999). Molecular basis of cardiovascular disease. Chien K.R. (ed). Saunders. Philadelphia. pp 393-410.

Navab M., Ananthramaiah G.M., Reddy S.T., Van Lenten B.J., Ansell B.J., Fonarow G.C., Vahabzadeh K., Hama S., Hough G., Kamranpour N., Berliner J.A., Lusis A.J. and Fogelman A.M. (2004). The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL. J. Lipid Res. 45, 993-1007.

Rodella L.F., Bonomini F., Rezzani R., Tengattini S., Hayek T., Aviram M., Keidar S., Coleman R. and Bianchi R. (2007). Atherosclerosis and the protective role played by different proteins in apolipoprotein E- deficient mice. Acta Histochem. 109, 45-51.

Schächinger V. and Zeiher A.M. (2002). Atherogenesis--recent insights into basic mechanisms and their clinical impact. Nephrol. Dial.

Transplant. 17, 2055-2064.

Shishehbor M.H., Brennan M.L., Aviles R.J., Fu X., Penn M.S., Sprecher D.L. and Hazen S.L. (2003). Statins promote potent systemic antioxidant effects through specific inflammatory pathways. Circulation 108, 426-431.

Sies H. (1985). Oxidative stress: introductory remarks. In: Oxidative stress. Sies H. (ed). Academic. New York. pp 1-8.

Sies H. (1991). Oxidative stress: oxidants and antioxidants. London: Academic.

Singh U. and Jialal I. (2006). Oxidative stress and atherosclerosis. Pathophysiology 13, 129-142.

Spickett C.M., Jerlich A., Panasenko O.M., Arnhold J., Pitt A.R., Stelmaszynska T. and Schaur R.J. (2000). The reactions of hypochlorous acid, the reactive oxygen species produced by myeloperoxidase, with lipids. Acta Biochim. Pol. 47, 889-899.

Spiekermann S., Landmesser U., Dikalov S., Bredt M., Gamez G., Tatge H., Reepschlager N., Hornig B., Drexler H. and Harrison D.G. (2003). Electron spin resonance characterization of vascular xanthine and NAD(P)H oxidase activity in patients with coronary artery disease: relation to endothelium-dependent vasodilation. Circulation 107, 1383-1389.

Stary H.C., Chandler A.B., Dinsmore R.E., Fuster V., Glagov S., Insull W. Jr., Rosenfeld M.E., Schwartz C.J., Wagner W.D. and Wissler R.W. (1995). A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Arterioscler. Thromb.

Vasc. Biol. 15, 1512-1531.

Steinberg D., Parthasarathy S., Carew T.E., Khoo J.C. and Witztum J.L. (1989). Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N. Engl. J. Med. 320, 915-924.

Stocker R. and Keaney J.F. Jr. (2004). Role of oxidative modifications in atherosclerosis. Physiol. Rev. 84, 1381-1478.

Stocker R. and Keaney J.F. (2005). New insights on oxidative stress in the artery wall. J. Thromb. Haemost. 3, 1825-1834.

Tilton R.G., Kawamura T., Chang K.C., Ido Y., Bjercke R.J., Stephan C.C., Brock T.A. and Williamson J.R. (1997). Vascular dysfunction induced by elevated glucose levels in rats is mediated by vascular endothelial growth factor. J. Clin. Invest. 99, 2192-2202.

Wassmann S., Wassmann K. and Nickenig G. (2004). Modulation of oxidant and antioxidant enzyme expression and function in vascular cells. Hypertension 44, 381-386.

Zhang R., Brennan M.L., Fu X., Aviles R.J., Pearce G.L., Penn M.S., Topol E.J., Sprecher D.L. and Hazen S.L. (2001). Association between myeloperoxidase levels and risk of coronary artery disease. JAMA. 286, 2136-2142.

Zouaoui Boudjeltia K., Moguilevsky N., Legssyer I., Babar S., Guillaume M., Delree P., Vanhaeverbeek M., Brohee D., Ducobu J. and Remacle C. (2004). Oxidation of low density lipoproteins by myeloperoxidase at the surface of endothelial cells: an additional mechanism to subendothelium oxidation. Biochem. Biophys. Res. Commun. 325, 434-438.