Review

Involvement of Oxidation-Sensitive Mechanisms in the Cardiovascular Effects of Hypercholesterolemia

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Hypercholesterolemia is a common clinical metabolic and/or genetic disorder that promotes functional and structural vascular wall injury. The underlying mechanisms for these deleterious effects involve a local inflammatory response and release of cytokines and growth factors. Consequent activation of oxidation-sensitive mechanisms in the arterial wall, modulation of intracellular signaling pathways, increased oxidation of low-density lipoprotein cholesterol, and quenching of nitric oxide can all impair the functions controlled by the vascular wall and lead to the development of atherosclerosis. This cascade represents a common pathological mechanism activated by various cardiovascular risk factors and may partly underlie synergism among them as well as the early pathogenesis of atherosclerosis. Antioxidant intervention and restoration of the bioavailability of nitric oxide have been shown to mitigate functional and structural arterial alterations and improve cardiovascular outcomes. Elucidation of the precise nature and role of early transductional signaling pathways and transcriptional events activated in hypercholesterolemia in children and adults, including mothers during pregnancy, and understanding their downstream effects responsible for atherogenesis may help in directing preventive and interventional measures against atherogenesis and vascular dysfunction.

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CHD = coronary heart disease; eNOS = endothelial nitric oxide synthase; HMG-CoA = 3-hydroxy-3-methylglutaryl coenzyme A; IL = interleukin; iNOS = inducible nitric oxide synthase; LAD = left anterior descending; LDL = low-density lipoprotein; NADPH = nicotine adenine dinucleotide phosphate; NFκB = nuclear factor κ B; NO = nitric oxide; NOS = nitric oxide synthase; ROS = reactive oxygen species

Progressive accumulation of evidence over the past 4 decades has shown that elevated plasma cholesterol is an independent risk factor for cardiovascular disease and increased mortality.[1] Establishment of the National Cholesterol Education Program in 1985 substantially increased the awareness of the risks associated with an elevated cholesterol level and provided guidelines for lipid-lowering therapy.[2] Despite a decline in mean serum cholesterol levels in the United States and the availability of therapeutic options, about 50% of middle-aged adults still have total cholesterol values higher than desirable,[3] which likely contribute to atherosclerosis being one of the major causes of coronary heart disease (CHD) and premature death in the United States.[4] Moreover, progressive lifestyle changes toward a Western diet with high-fat intake are increasing the incidence of hypercholesterolemia[5,6] and consequently CHD[7] in populations that previously had a relatively low risk for the disease.

MECHANISMS UNDERLYING CARDIOVASCULAR EFFECTS OF HYPERCHOLESTEROLEMIA

The increased availability of lipids in hypercholesterolemia promotes initiation and progression of atherogenesis in the arterial wall because the oxidative modification and uptake of lipids are not feedback controlled, thus facilitating their excessive uptake.[8] Indeed, angiographic trials have demonstrated that cholesterol-lowering therapy significantly reduced progression and increased regression of atherosclerotic coronary arterial lesions.[9] Surprisingly, however, the marked reduction in clinical events far outweighed the relatively modest frequency and magnitude of lesion regression, which may be partly explained by local depletion of lipids and plaque stabilization, with an ensuing decrease in acute coronary events.[10] Furthermore, the increased incidence of cardiac events associated with hypercholesterolemia in the absence of significantly obstructive CHD has been partly attributed to functional alterations in the arterial wall, such as impaired coronary vascular responses[11] to various stimuli, and to increased cardiac demand.[12,13] Indeed, over the past 2 decades it has become apparent that endothelium-dependent vascular relaxation is abnormal in

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various disease states, including hypercholesterolemia, atherosclerosis, diabetes mellitus, hypertension, and preeclampsia, and after heart transplantation.[14-16]

In 1986 Ludmer et al[17] first demonstrated that intracoronary infusion of acetylcholine in humans with CHD caused coronary vasoconstriction. Altered vasomotor regulation by the endothelium has subsequently been shown to be an early development of atherogenesis[18] involving both large vessels and the microcirculation[19] and to be at least partly reversible with lipid-lowering therapy,[20] blockade of the renin-angiotensin system,[21] and/or antioxidant therapeutic strategies.[14] Nonetheless, the abnormal regulation of vascular tone may reflect impairment in an array of functions controlled by the vascular wall, such as lipid breakdown, platelet function, coagulation, monocyte adhesion, inflammation, and vessel growth.[22,23] The underlying mechanisms responsible for these abnormalities are likely multifactorial, but major contributors that appear to be enhanced during hypercholesterolemia and could be responsible for the early endothelial dysfunction include (1) uncoupling of receptors from G proteins, (2) increased production of oxygen radicals, and (3) decreased availability of L-arginine, a nitric oxide (NO) precursor, coupled with reduced NO production by the damaged endothelium (see subsequent discussion). Indeed, several such events may stem from activation of redox-sensitive mechanisms involved in cellular signaling[24-28] (such as nuclear factor κB [NFκB][27,29] and c-Myc[30]), increased levels, and subsequent oxidation of low-density lipoprotein (LDL) and oxidative degradation of NO.

Reactive Oxygen Species

A growing body of evidence suggests that numerous pathological conditions are associated with an increased vascular (and to a lesser extent plasma) production of reactive oxygen species (ROS) and other radicals,[14,31] the most prominent of which are superoxide and hydroxyl radical,[32] and oxidants like hydrogen peroxide and peroxynitrite.[33] This form of pro-oxidant shift in vascular redox status (the so-called oxidant stress), particularly interactions between NO and ROS, represents a common pathological mechanism activated by many cardiovascular risk factors.[31] Furthermore, ROS seem to serve important cellular signaling mechanisms responsible for many of the features of vascular dysfunction and atherogenic lesion formation.[25,28,31]

Hypercholesterolemia per se may enhance formation of lipid peroxidative compounds, which are formed when ROS (and/or other radicals) react with increased levels of plasma and tissue lipids. The precipitating events in radical generation during the evolution of atherosclerosis appear to involve early injury to the vascular endothelial layer,[34] which increases its adhesiveness, permeability, and procoagulation properties, and damage to the intima via formation of oxidized lipoproteins (see subsequent discussion).[35] Penetration and accumulation in the arterial wall of triglyceride-rich lipoproteins, including very LDL, chylomicrons, and their remnants, activate or induce the synthesis of factors that can initiate inflammatory responses. These include plasminogen activator inhibitor 1 (which may interfere with fibrinolysis), protein kinase C, mitogen-activated protein kinase, and NFκB, which has an important role in the phenotypic modulation of endothelial cells to a proinflammatory condition.[36] In particular, LDL has numerous effects on the endothelium, including those on plasminogen activator inhibitor 1 and induction of adhesion molecule expression.[36] Native LDL per se can induce the release of superoxide anion,[37] leading to formation of the potent oxidant peroxynitrite and triggering a vicious cycle of oxidation. Subsequent oxidation of LDL yields its oxidized form, which possesses greater efficacy in initiation of superoxide anion production and endothelial dysfunction.[38] Thus, early injury induces the endothelium to have procoagulant properties; to form vasoactive molecules, cytokines, and growth factors; and to initiate a local inflammatory response that might continue indefinitely.[35]

Release of cytokines and growth factors,[35] such as tumor necrosis factor α, interleukin (IL)-1β, and interferon-gamma, may in turn stimulate ROS-producing enzymes like nicotine adenine dinucleotide phosphate (NADPH) oxidase, the major source of superoxide anion in vascular cells and myocytes,[39] xanthine oxidase,[40,41] NO synthase (NOS), cyclooxygenase, myeloperoxidase,[42] and lipoxygenase.[43] This cascade may also involve local activation of the renin-angiotensin system,[44] which can promote oxidation via angiotensin II-induced stimulation of NADPH oxidase.[45] The vascular NADPH oxidase is similar in structure to the neutrophil NADPH oxidase,[32] and it produces superoxide and hydrogen peroxide on stimulation by mechanical forces (eg, stretch and shear stress),[46] hormonal stimulation,[45] or growth factors and cytokines.[39] In hypercholesterolemia an important role for increased production of ROS is also ascribed to xanthine oxidase,[47] which, due to an increase in plasma cholesterol, is released into the circulation where it binds to endothelial cells and sustains production of superoxide anion.[47]

The cellular sources of these species include blood-borne phagocytic cells and infiltrating monocytes (Figure 1), in addition to cells within the vascular wall, such as smooth muscle cells, endothelial cells, and fibroblasts.[48] Notwithstanding the presence of macrophages and the postulated involvement of superoxide in atherogenesis, its NADPH oxidase-derived source may be from vascular cells because paucity of phagocytic NADPH oxidase does not decrease lesion size in apolipoprotein E- and gp91-

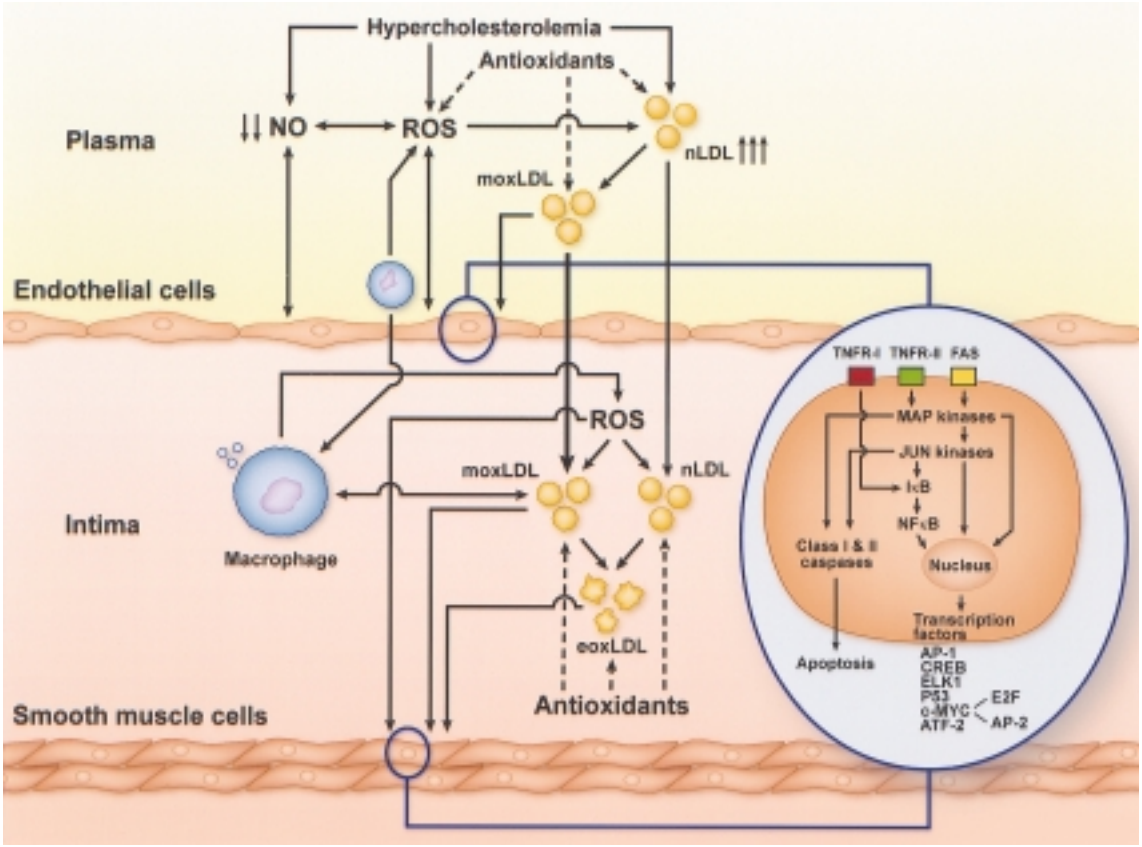

Figure 1. Oxidation-sensitive mechanisms activated in the arterial wall during hypercholesterolemia. Increased availability of native low-density lipoprotein (nLDL) cholesterol and release of reactive oxygen species (ROS) lead to production of "minimally" or "mildly" oxidized LDL (moxLDL) in association with a decrease in bioavailability of nitric oxide (NO). These alterations may interfere with the normal function of the blood vessel and thus induce endothelial dysfunction. Furthermore, increased uptake of moxLDL by the vascular wall results in further oxidation of LDL to an "extensively" modified (eoxLDL) form and subsequent accumulation and macrophage uptake of LDL, which in turn promote inflammatory responses and continued release of ROS. Pro-oxidant effects on endothelial and smooth muscle cells initiate a chain reaction of redox-sensitive transductional and transcriptional events (inset). Activation of both FAS and tumor necrosis factor receptors (TNFR) and the caspase, mitogen-activated protein (MAP) kinase, and JUN kinase pathways lead to stimulation of various oxidation-sensitive transcription factors that have the potential to increase the expression of various cell adhesion molecules and inflammatory gene products. Antioxidants can interfere with this pathophysiological scenario at several points by scavenging or blunting the release of ROS and by retarding LDL oxidation. AP = activator protein; ATF = activating transcription factor; CREB = cyclic adenosine monophosphate response element binding protein; E2F = elongation 2 factor; ELK = ets-like element kinase; IκB = inhibitor of κ B; NFκB = nuclear factor κ B.

phox subunit deficient knock-out mice.[49] Although cells are normally protected from ROS by antioxidant defense mechanisms, such as the oxygen-radical scavenger enzymes catalase, superoxide dismutase, and glutathione peroxidase, the rate of ROS formation can exceed the antioxidant defense capacity and thereby increase oxidant stress.[43] The precise mechanisms whereby cells in the arterial wall produce ROS are only presently coming to light and will almost certainly prove to be a primary focus of future therapeutic strategies.

The ROS in turn initiate a chain reaction of redox-sensitive signaling events through which they influence vascular smooth muscle cell growth and migration, modulation of vascular function, expression of a proinflammatory phenotype, and modification of the extracellular matrix[32] (Figure 1). The signaling pathways through which ROS exert these effects include activation of both Fas and tumor necrosis factor receptors,[28] caspase, and the mitogen-activated protein kinase/Jun kinase pathways.[24] As a result, several redox-sensitive transcription factors are stimulated, such as activating transcription factor 2, ets-like element kinase-dependent 1, cyclic adenosine monophosphate response element binding protein, NFκB, activator protein 1 complex, p53, and c-Myc/Max complex and its binding

factors elongation 2 factor and activator protein 2 complex[24,25,28,29] (Figure 1). This can lead to increased expression of various cell adhesion molecules and inflammatory gene products, such as vascular cell adhesion molecule 1, intercellular adhesion molecule 1, and monocyte chemoattractant protein 1. The involvement of ROS in activation of these pathways is underscored by the successful attenuation of these components using antioxidants, both in vitro and in vivo,[24,25,43] and oxygen-radical scavengers.[28] Accumulating evidence also provides a compelling case for enhanced oxidative stress in vascular dysfunction, the most important manifestation of which is modulation of a set(s) of proinflammatory genes that are regulated directly or indirectly by ROS in the arterial wall.[50]

Viewed in this perspective, LDL oxidation could be an important consequence of a generalized metabolic oxidation-related abnormality of the arterial wall in atherosclerosis, rather than a single core pathophysiological feature. The fact that hypercholesterolemia, hypertension, and advanced glycosylation end products formation linked to diabetes mellitus all activate similar redox-sensitive proinflammatory genes associated with the pathogenesis of atherosclerosis and decrease the bioavailability of NO provides the potential for the development of a unifying framework concerning the etiology of atherosclerotic disease.[16,35,51,52]

The atherogenic effect of hypertension may be partly mediated via activation of the renin-angiotensin system, stimulation of NADPH oxidase,[45] and increased levels of proinflammatory genes in the vessel wall[53]; however, shear stress and vascular hemodynamic stress per se can also trigger redox-sensitive mechanisms and thus contribute to hypertension-induced atherogenesis.[46,54] Diabetes mellitus and hyperglycemia also augment generation of superoxide anions and LDL peroxidation,[55] promote activation of redox-sensitive pathways,[56] decrease bioavailability of NO,[57] induce formation of advanced glycosylation end products, and induce endothelial dysfunction.[58] Indeed, activation of intracellular oxidative signals and modulation of vascular proinflammatory gene expression may provide a molecular mechanism underlying the synergism among cardiovascular risk factors and the early pathogenesis of atherosclerosis.[43] These concepts also underscore the potential of antioxidants as attractive therapeutic agents.[24,25,59]

The effects of experimental hypercholesterolemia and oxidation-sensitive mechanisms on this cascade of events have been investigated in various animal models, such as murine[60-62] and swine.[63,64] Experimental investigation of the development and progression of atherosclerosis has been greatly facilitated by the use of targeted mouse models of the disease, particularly those resulting from the absence of functional genes.[65] Mice are rapidly becoming a ubiquitous model of atherogenesis, and although wild-type mice are inherently resistant to hypercholesterolemia and atherogenesis, identification of genes determining the susceptibility to the disease and gene-knockout and transgene approaches have led to generation of the apolipoprotein E knockout mouse, LDL-receptor deficient mouse, and double-knockout mouse models that develop extensive atherosclerosis.[60-62] The hypercholesterolemic mouse model also facilitated study of the "priming" phenomenon, which involves activation of NFκB-dependent genes in lesion-prone areas.[66]

The pig model is appealing for studying human disease states because the anatomy and physiology of the pig heart are comparable to those of humans.[67] In hypercholesterolemic pigs, coronary vasomotor dysfunction is associated with an increase in both the level[64] and the vasoconstrictor effects[68] of endothelin-1, as well as decreased bioavailability of NO,[68,69] activation of NFκB,[29] and priming of c-Myc.[30] Increased vasoconstrictor activity of oxidation byproducts in this model[70] can be blunted by endothelin receptor blockade.[71,72] These changes were associated with alterations in intramyocardial microvascular function[73] and spatial density.[74] Indeed, we recently showed[75] that in this model hypercholesterolemia was associated with blunted myocardial perfusion and increased vascular permeability responses to increased cardiac demand, accompanied by depletion of tissue endogenous radical scavengers and enhanced oxidizability of LDL, all of which were correctable with long-term antioxidant therapy. We subsequently showed that similar oxidation-sensitive mechanisms were activated in the systemic and renal circulation of the renovascular hypertensive pig model[15] and markedly augmented by coexistence with hypercholesterolemia,[16] paralleled by renal perfusion abnormalities. A growing bulk of evidence shows that coronary blood flow and myocardial perfusion are altered in humans with hypercholesterolemia[76-79] and are clinically consequential[11]; they also may be related to the concurrent oxidative stress.

Oxidized LDL

Susceptibility to atherosclerosis is associated with elevations in specific populations of apolipoprotein B–containing particles involving increased oxidation of LDL and associated changes in its biological properties.[80,81] Lipoprotein oxidation may be potentiated by the greater mass of oxidizable LDL substrates available in hypercholesterolemia, by a decrease of natural antioxidants bound on LDL, and by a greater intrinsic susceptibility for oxidation of the specific steric forms of LDL (eg, small dense LDL) that arise in these disorders.[80,81] Entrapment of the LDL particles within the arterial wall (Figure 1) results in progressive oxidation (to minimally and extensively oxidized

forms) and internalization of this modified LDL by an entire family of scavenger receptors on macrophages and smooth muscle cells, which in turn lead to formation of lipid peroxides, activation of inflammatory cytokines, and a vicious cycle of inflammation and oxidation, eventuating in accumulation of lipid-laden foam cells and plaque formation.[35] The broad cascade of transduction and transcriptional events in the arterial wall induced by 2 forms of oxidized LDL is shown in Figure 1. These pathways activated by minimally and extensively oxidized LDL are probably largely similar to those activated by ROS and other radicals.[24,28] Obviously, several "intermediate" forms of oxidized LDL can be formed in the intima with different degrees of both lipid and protein oxidation. Considerable light has been shed on the mechanisms of interaction between hypercholesterolemia and hypertension with the recent discovery of LOX-1,[82] a novel lectin-like receptor for oxidized LDL on vascular wall cells that is inducible by angiotensin II[83] and fluid shear stress,[84] is up-regulated in both disease states,[85,86] and may provide an additional link between hypertension and atherogenesis.[87] Furthermore, the ability of oxidized LDL to up-regulate its own endothelial receptor[88] provides an additional route for the vicious cycle of pathophysiological events triggered in hypercholesterolemia.

These facets of LDL oxidation are potentiated in several forms of dyslipidemia and, in view of both differences and similarities among these disorders, indicate that LDL oxidative behavior is determined by a complex array of physical, chemical, and metabolic factors.[89-91] Moreover, long-term residence of circulating LDL and aging may increase LDL's susceptibility to further oxidative modification in the arterial wall.[92]

Oxidized LDL exerts profound effects on the vasomotor response of isolated arteries to various stimuli that closely mimic the vascular dysfunction associated with hypercholesterolemia and atherosclerosis in humans.[80,93] The inhibitory effect of oxidized LDL on vascular relaxation may be related to uncoupling of endothelial receptors from endothelial guanine nucleotide-binding regulatory (Gi) protein and interruption of Gi protein-dependent pathways.[94] The beneficial effect of lipid-lowering therapy in restoring vascular function[95] and greatly decreasing the frequency of cardiac events associated with atherosclerosis,[96] combined with the ability of antioxidants to alleviate vasomotor disturbances in hypercholesterolemia and slow the progression of atherosclerosis, strongly supports a causative role of oxidized LDL in mediating vascular dysfunction in vivo and contributing to both preclinical and clinical sequelae of CHD.[93] Further research for a more complete understanding of the mechanisms of oxidized LDL formation and actions in vivo may reveal novel strategies to inhibit these pathophysiological events and may prove beneficial in the therapeutic management of atherosclerosis-related diseases.

Nitric Oxide

One of the main mechanisms underlying impaired endothelial function in hypercholesterolemia and other cardiovascular risk factors[97] is decreased bioavailability of NO.[26] Bioavailability of NO is probably impaired not by a single defect but by various mechanisms affecting both its synthesis and its breakdown,[26,98] among which increased superoxide anion production and oxidative stress represent major mechanisms.

Endogenous NO is generated by a family of 3 distinct calmodulin-dependent NOS enzymes. The endothelial (eNOS) and neuronal NOS isoforms are both constitutively expressed enzymes whose activities are stimulated by increases in intracellular calcium. In the endothelium, eNOS converts the amino acid L-arginine to L-citrulline and NO, a reaction that requires availability of both the substrate (L-arginine) and a cofactor, tetrahydrobiopterin, which couples L-arginine oxidation to NADPH consumption.[99] Tight control of NO signaling is largely regulated at the level of eNOS biosynthesis,[100] which can be achieved by modulation of eNOS gene expression (eg, by shear stress or cell proliferation) or activity (eg, by shear stress or stimulation of specific receptors to agonists). Various stimuli for eNOS also alter cellular redox state, suggesting that ROS might modulate eNOS expression.[101] Localization of eNOS to specialized plasma membrane invaginations (caveolae) seems to be required for its maximal activity,[102] and phosphorylation and subcellular translocation (from caveolae to the cytoskeleton or cytosol) are probably involved in eNOS regulation.[103] Recent evidence indicates that, in hypercholesterolemia, increased cholesterol uptake by endothelial cells up-regulates the abundance of the structural protein and signal transduction regulator caveolin-1[104] (a putative negative regulator of eNOS[105,106]) and stabilizes the inhibitory heterocomplex that it forms with eNOS.[105] Similarly, ROS decrease both eNOS expression and association with caveolin.[102] Furthermore, in the absence of either L-arginine or tetrahydrobiopterin (eg, due to tetrahydrobiopterin oxidation by peroxynitrite), eNOS produces superoxide and hydrogen peroxide,[107] a phenomenon termed *NOS uncoupling*.[108] In fact, eNOS uncoupling may contribute to increased oxidative stress by both decreasing generation of NO and increasing release of superoxide.

Immune functions of NO are mediated by a calcium-independent inducible NOS (iNOS).[100] This isoform of the NOS enzyme is often involved in proinflammatory processes[109]; is expressed in inflammatory, smooth muscle, and endothelial cells of human atherosclerotic lesions[110];

and is especially prominent in macrophages.[111] Its role in atherogenesis is controversial because it appears that iNOS-derived NO can inhibit, have no effect, or enhance leukocyte rolling and adhesion depending on the type of inflammatory response.[112] Indeed, concurrent iNOS deficiency reduced the size of atherosclerotic lesions in atherosclerosis-susceptible apolipoprotein E–deficient mice,[109] and its inhibition limited progression of preexisting atherosclerosis in hypercholesterolemic rabbits[113]; however, iNOS also counteracted progression of intimal thickening during periadventitial inflammation in rabbit carotid artery[114] and inhibited inflammatory cytokine-induced proliferative and vasospastic changes in porcine coronary artery in vivo.[115] Interestingly, iNOS-derived NO inhibits eNOS in rat kidney and provokes renal dysfunction consequent to lipopolysaccharide administration.[116] Further studies are needed to define more precisely the conditions under which iNOS exerts beneficial or detrimental effects on the development of atherosclerosis.

Modulation of NO-dependent pathways could provide several benefits in negating atherosclerotic lesion formation and progression.[117] Among the normal functions of NO are inhibition of platelet adherence and aggregation, reduction in adherence of leukocytes to the endothelium, and suppression of vascular smooth muscle cell proliferation.[118] NO is a potent antioxidant, ROS scavenger, and modulator of inflammatory and signal transduction pathways, which can modulate lipid peroxidation and proinflammatory gene expression.[119,120] However, NO and its products, such as reactive nitrogen species, may also exert pro-oxidant effects, like increase membrane and lipoprotein lipid oxidation and foam cell formation. This type of reaction depends on the relative concentrations of NO, ROS, and antioxidants, as well as on the aqueous-lipid solubility and relative rates of reaction of the participating reactive species. Hence, when endogenous tissue rates of oxidant production are accelerated or when tissue oxidant defenses are depleted, NO-derived oxidizing species can promote proatherogenic effects.[121] Therefore, a decrease in the relative bioavailability of NO not only impairs endothelium-dependent vasodilation but also activates other mechanisms that have an important role in the pathogenesis of atherosclerosis.[122]

Patients with hypercholesterolemia have impaired receptor- and endothelium-dependent vascular relaxation, which may predispose coronary arteries to vasoconstriction.[123] Loss of basal and flow-mediated NO production has been shown in the proximal segments of coronary arteries,[124] while the vasorelaxant effect of nitroglycerin appears to be preserved,[125] although not in all vascular beds.[126] The physiological role of NO in the regulation of vascular tone by the endothelium is diminished in patients with hypercholesterolemia.[127] Obviously, therapeutic strategies that aim to improve the bioavailability of NO in these patients are warranted.[26]

NOVEL TREATMENT STRATEGIES IN HYPERCHOLESTEROLEMIA

Hypercholesterolemia plays a major causal role in atherogenesis, and therefore reduction of blood cholesterol is a primary therapeutic target. Progress has been substantial in identifying pathogenic mechanisms of atherosclerosis, in particular with regard to the role of apolipoproteins and scavenger receptors, adhesion molecules, growth factors, and interleukins.[14,35,52,128] Both primary[96] and secondary[129,130] prevention trials have demonstrated unequivocally the decrease in coronary morbidity and mortality that can be achieved by lowering lipids in patients with hypercholesterolemia. To decrease the rate of cardiac events and mortality, the target goals of therapy are generally to achieve LDL levels of 100 mg/dL or lower in patients with established CHD and 130 mg/dL or lower in high-risk patients without established CHD. Conventional interventions include lifestyle modification (eg, reduced intake of saturated fatty acids and cholesterol and increased physical activity), addressing other concurrent cardiovascular risk factors (such as obesity, hypertension, diabetes, or postmenopause), and drug therapy.

Lipid-lowering drugs include bile acid sequestrants, nicotinic acid, and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors ("statins"), all of which have important roles in cholesterol-lowering therapy, alone or in combination.[3] The agents with the greatest LDL cholesterol-lowering effect are the bile acid sequestrants, which up-regulate the LDL receptor by interruption of enterohepatic circulation of cholesterol-rich bile acids, and the statins, which interfere with the cholesterol biosynthesis pathway by inhibiting HMG-CoA reductase.[131] The greatest triglyceride-lowering effect is exerted by nicotinic acid, which decreases the production of very LDL cholesterol and reduces the availability of circulating free fatty acids, and the fibric acid derivatives, which activate hepatic peroxisome proliferator-activated receptor α-1 and lipoprotein lipase, thus improving the plasma transport rates of several lipoproteins, and inhibit inflammatory mediators.[132] Among these, statins are the most commonly prescribed agents for the treatment of hypercholesterolemia because of their efficacy in reducing both LDL levels and event rates and because of their excellent tolerability and safety.[133] When this approach does not achieve desired goals in LDL levels, in rare circumstances (such as familial hypercholesterolemia), patients can undergo LDL apheresis, which has been associated with a decreased CHD event rate.[134]

Remarkably, some of the conventional interventions exert their beneficial effects to some extent through attenuation of oxidation-sensitive mechanisms. For example, long-term exercise training may improve bioavailability of NO and endothelium-mediated vasorelaxation,[135] partly by increasing expression of CuZn superoxide dismutase[136]; however, this mechanism does not appear to have a major role in acute exercise-induced skin and muscle hyperemia.[137] Notwithstanding an evident need to decrease plasma cholesterol, novel strategies in recent years have attempted to target mechanisms responsible for the outcomes of hypercholesterolemia, with primary goals of both decreasing oxidant stress and increasing bioavailability of NO.

Antioxidant Approaches

The large body of evidence supporting the important contribution of endogenous oxidative stress to the development of early atherosclerosis in humans[138-140] has led to the use of antioxidant therapy in an attempt to prevent functional and structural vascular damage. In vitro, cellular, and animal studies clearly demonstrate that vitamin E, the most important fat-soluble antioxidant, and vitamin C, a highly potent water-soluble antioxidant,[141] can retard LDL oxidation and protect animals against various types of oxidative stress,[25,59,142] and they can potentially interrupt the downstream sequence of atherogenic events. Clinical intervention trials with long-term antioxidant therapy support the notion that supplemental vitamin E can reduce the risk of myocardial infarction and heart disease,[143] although results regarding improved cardiac outcomes in high-risk patients with atherosclerosis are occasionally conflicting.[144] Indeed, the efficacy of antioxidant treatment is likely related to the duration, timing, type, and dose of dietary supplementation.[59] In particular, the combination of vitamin E (400-1200 IU/d) with moderate doses of vitamin C (ascorbate), which scavenges a wide range of reactive radicals (oxygen, nitrogen species, and others) and regenerates vitamin E,[145] appears to be effective in reducing lipid peroxidation in humans, both in vitro and in vivo,[146] without major adverse effects.[59,147]

Notably, the efficacy of the widely prescribed HMG-CoA reductase inhibitors in decreasing the incidence of cardiac events and mortality rate is likely enhanced by their possible antioxidant properties,[55,148] which may underlie their capacity to improve vascular function[95] and plaque morphology[149] independent of lowering of lipids. Recent evidence indicates that statins also inhibit vascular smooth muscle cell growth[150] but activate the protein kinase AKT to promote new blood vessel growth,[151] which may serve as an additional beneficial mechanism to inhibit plaque formation.[152] Their capability to reduce the expression of IL-1β and IL-6, peroxisome proliferator-activated receptor α and γ, and the p22-phox and p47-phox subunits of NADPH oxidase underscores their beneficial anti-inflammatory and antioxidant attributes.[153] Moreover, vitamin E supplementation benefits endothelial function beyond that achieved with statins alone.[154]

In extreme increases in blood cholesterol, LDL apheresis is a safe procedure for patients with homozygous familial hypercholesterolemia[90,91] or severe CHD.[155] During selective LDL apheresis, an increase in plasma glutathione concentrations was observed, which was unaccompanied by a significant reduction in the plasma activity of antioxidant enzymes, LDL, red blood cells, or granulocytes and may explain the lack of plasma lipid peroxidation shown during this kind of extracorporeal treatment.[90,155] In addition, LDL isolated at the end of apheresis procedures is more resistant to oxidation.[90]

Restoration of NO Bioavailability

Endothelial dysfunction contributes to the pathogenesis of myocardial ischemia, and cholesterol-lowering therapy may restore vascular function.[156] Moreover, since depletion of L-arginine may contribute to endothelial dysfunction, infusion of L-arginine improves the forearm resistance vessel blood flow responses to methacholine in patients with hypercholesterolemia.[157] Administration of L-arginine partially restores endothelium-dependent vasodilation in patients with hypercholesterolemia[158,159] and dilates coronary stenoses in patients with CHD.[160] L-arginine supplementation for 6 months in humans also improves coronary small-vessel endothelial function, in association with a significant improvement in symptoms,[161] and may be a therapeutic option for patients with coronary endothelial dysfunction and nonobstructive CHD.[161] Indeed, oral L-arginine led to a significant clinical improvement in 70% of patients with intractable angina pectoris, in association with a significant decrease in cell adhesion molecule and proinflammatory cytokine levels,[162] suggesting that it may also have anti-inflammatory properties. The mechanisms by which NO bioavailability can be improved with any drug therapy remain to be elucidated and may provide further insights into the mechanisms involved in impaired endothelial function and atherogenesis.[117]

EFFECTS OF HYPERCHOLESTEROLEMIA AND INCREASED OXIDATION ON EARLY ATHEROGENESIS IN HUMANS

Until recently, atherogenesis was thought to begin during late childhood, although fatty streaks had occasionally been observed in younger children.[163,164] However, a systematic morphometric analysis of the entire aorta of premature human fetuses demonstrated that formation of fatty

streaks, the precursors of more advanced atherosclerotic lesions, is prevalent in all fetal aortas and that their number and size are markedly increased in fetuses whose mothers had hypercholesterolemia during pregnancy.[138] Fetal lesions contained typical components of early atherosclerotic lesions, such as native and oxidized LDL and macrophages, and their distribution reflected that of more advanced atherosclerosis seen in adults, ie, most extensive in the abdominal aorta, followed by the aortic arch. This suggests that, during the earlier stages of pregnancy, maternal hypercholesterolemia may promote early atherogenesis in the fetus.[165,166] The assumption that LDL oxidation is a contributor to atherogenesis in fetal arteries was also supported by a later study[167] in which the middle cerebral and basilar arteries of fetuses contained significantly smaller lesions than the aorta and common carotid arteries. Determinations of the arterial activities of oxygen-radical scavengers, such as manganese superoxide dismutase, catalase, and glutathione peroxidase, indicated that overall intracranial arteries of human fetuses were better protected against oxidation than extracranial arteries.[167] These results are consistent with the assumption that better protection against free radical–mediated oxidation may contribute to the greater resistance of intracranial arteries to hypercholesterolemia-induced atherogenesis and vascular dysfunction.[168]

To investigate whether fetal lesions regress and/or whether they influence atherogenesis during childhood and adolescence, the Fate of Early Lesions in Children (FELIC) study was designed.[139] Atherosclerosis was established by computer-assisted image analysis in normocholesterolemic children and was found to progress much faster in children whose mothers had hypercholesterolemia during pregnancy than in children of normocholesterolemic mothers, despite normal lipid profiles in both groups of children. None of the risk factors of atherogenesis assessed in these children could account for the faster atherogenesis in children of hypercholesterolemic mothers. Although parental genetic differences are likely to contribute to the different susceptibility of children to the disease, we postulated that maternal-fetal hypercholesterolemia induced constitutive changes in gene expression in arterial cells, which were associated with a greater susceptibility to the disease later in life.[165,166] A recent study demonstrated that fetal lesions in the rabbit can be reduced with vitamin E or cholestyramine treatment of the hypercholesterolemic mothers during pregnancy.[169]

Maternal hypercholesterolemia during gestation should therefore be added to the list of risk factors determining the need for monitoring and for preventive therapy.[170] Current clinical guidelines place great emphasis on early detection of hypercholesterolemia,[171] although such screening would not detect an increased risk associated with maternal hypercholesterolemia in normocholesterolemic subjects. An intense lipid-lowering intervention may be a therapeutic option for children with several risk factors. As indicated by a recent meta-analysis of studies on the development of coronary artery disease in children and adolescents,[172] an average reduction of LDL cholesterol by 25% can be obtained with statins in combination with a lipid-lowering diet. Statins are generally well tolerated in children and adolescents, and current data do not indicate adverse effects on growth and sexual development in male adolescents. In high-risk children, follow-up may need to include an attempt for an earlier than usual noninvasive diagnosis of atherosclerosis. Potential approaches include measurement of coronary flow velocity in the distal left anterior descending (LAD) coronary artery with transthoracic Doppler echocardiography,[173] determination of the degree of stenosis in the proximal LAD coronary artery by transesophageal Doppler study[174] or magnetic resonance imaging,[175] measurement of coronary flow reserve in the LAD coronary artery by contrast-enhanced transthoracic second harmonic echocardiography/Doppler study,[176] or quantification of coronary calcifications by electron beam computed tomography.[177-179]

Another clinical scenario that may involve dyslipidemia and oxidative stress is the pregnancy-related preeclampsia syndrome, the etiology and pathogenesis of which remain poorly understood.[180] Recent evidence points to a pro-oxidant shift in preeclampsia, and ROS and/or their metabolites have been hypothesized to ultimately compromise the vasodilatory, antiaggregatory, and barrier defense functions of the endothelium. Failure of flow-induced shear stress may contribute to the gestational hypertension of preeclampsia.[181] Maternal dyslipidemia and altered iron kinetics in preeclampsia may potentially affect disease progression.[180] Oxidative stress as a result of interaction of fetoplacental and maternal factors and autoimmune reaction may lead to the manifestations of preeclampsia. For example, interaction of maternal neutrophils and oxidized lipids with placental cells and placenta-derived factors can engender a vicious cycle of oxidative stress that may ultimately cause widespread endothelial cell dysfunction and physiological perturbations downstream of cellular signaling. A randomized controlled trial recently showed that vitamin C and E supplementation may be beneficial in women with or at increased risk for preeclampsia,[182] suggesting that the “primum movent” of the disease was increased lipid oxidation during pregnancy.

CONCLUSIONS

Hypercholesterolemia is a common clinical disorder that may begin early in life in humans, and it subsequently promotes atherogenesis by injuring the vascular wall,

thereby impairing a multitude of functions and signaling pathways that it controls and leading to development of atheromatous plaques. The underlying mechanisms responsible for these abnormalities may emanate from activation of oxidation-sensitive mechanisms, increased oxidation of LDL cholesterol, and quenching of NO. This cascade of events can begin as early as during pregnancy, altering the complex framework of signaling network in the arterial wall. Novel treatment strategies that attempt to decrease oxidation and restore bioavailability of NO have the potential to decrease morphologic and functional arterial damage and improve cardiovascular outcomes in patients with hypercholesterolemia.

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