The Role of Myeloperoxidase in HDL Oxidation and Atherogenesis

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High-density Lipoprotein–mediated Cholesterol Efflux

High-density lipoprotein (HDL) protects the artery wall against the development of atherosclerosis [1,2]. This atheroprotective effect is attributed in part to the ability of HDL to mobilize excess cholesterol from arterial macrophages. HDL components can remove cellular cholesterol by multiple mechanisms. Two of these processes involve cell membrane ATP-binding cassette transporters (ABCA1 and ABCG1) that are highly induced in macrophages when they accumulate too much cholesterol [1–4]. ABCA1 mediates the transport of cholesterol and phospholipids from cells to lipid-poor apolipoproteins [1,2], whereas ABCG1 mediates the transport of cell cholesterol to lipidated HDL particles and other lipoproteins [3,4]. Studies with atherosclerosis-susceptible mouse models have shown that ablation of either of these genes leads to accumulation of cholesterol in tissue macrophages.

Antioxidant Properties of HDL

HDL is the major carrier of lipid hydroperoxides in plasma in both humans and animal models of atherosclerosis [5]. Enzymes carried by HDL, including paraoxonase-1 (PON-1), lecithin-cholesteryl ester acyltransferase (LCAT), and lipoprotein-associated phospholipase A2, have been proposed to degrade lipid oxidation products [6]. Moreover, HDL hydroperoxides are reduced to corresponding hydroxides in concert with conversion of apolipoprotein A-I (apoA-I) methionine residues to methionine sulfoxide [7]. Cholesteryl ester hydroperoxides in HDL may be rapidly and selectively removed by liver cells [8]. Collectively, these observations suggest that antioxidant effects of HDL may make important contributions to the lipoprotein's antiatherogenic and anti-inflammatory properties.

Pathways for Oxidative Damage in the Human Artery

Oxidative damage of HDL apolipoproteins in the artery wall may promote atherogenesis by impairing cholesterol efflux from macrophages [9]. The activated macrophage is a leading suspect in lipoprotein oxidation. Moreover, mononuclear cells have enzymes such as myeloperoxidase (MPO) that produce an array of reactive species [10]. MPO has been identified as one pathway for oxidative damage in the human artery wall [11–13], and we have developed mass spectrometric (MS) techniques for quantifying amino acid oxidation products in vivo [14].

MPO Is Expressed in Human Atherosclerotic Lesions

To test the hypothesis that MPO might oxidize lipoproteins in vivo, we first searched for evidence that the enzyme is present in human atherosclerotic lesions [11]. Immunostaining for MPO and CD68, an epitope specific for monocytes and macrophages, demonstrated that MPO is expressed in human atherosclerotic lesions, predominantly in macrophages. Moreover, we used isotope dilution mass spectrometry to show that levels of 3-chlorotyrosine, a specific product of MPO, are markedly increased in low-density lipoprotein (LDL) isolated from human atherosclerotic lesions [15,16]. These observations, together with the detection of other amino acid oxidation products generated by MPO in vitro [17,18], provide strong evidence that MPO is enzymatically active in human atherosclerotic tissue and that LDL is one target for damage.

In vitro, MPO disappears as monocytes differentiate, and it is generally thought to be absent from macrophages [19]. However, the striking co-localization of immunostaining for MPO and macrophages that has been observed in transitional lesions [11] raises the possibility that lipid-laden macrophages, the cellular hallmark of the atherosclerotic lesion, continue to express MPO in vivo in response to cytokines or other regulatory factors. Indeed, in collaborative studies with Dr. Peter Libby (Harvard Medical School), we showed that human monocytes cultured with granulocyte macrophage colony-stimulating

factor continue to express MPO as they differentiate into macrophages [19].

With Dr. Renee LeBoeuf (University of Washington), we generated a transgenic mouse that expresses human MPO selectively in macrophages [20]. Transplantation of bone marrow from these mice into LDL receptor-/- (LDLR-/-) mice fed a high-fat, high-cholesterol diet significantly increased atherosclerosis when compared with mice transplanted with bone marrow from either wild-type or LDLR-/- mice. These results strongly support the hypothesis that MPO derived from macrophages is atherogenic in this mouse model of hyperlipidemia [20].

HDL Is Chlorinated and Nitrated in Humans Suffering from Established Cardiovascular Disease

The major product of MPO is generally thought to be hypochlorous acid (HOCl), which converts tyrosine to chlorotyrosine.

$$\text{HOCl} + \text{Tyrosine} \rightarrow \text{3-Chlorotyrosine} + H_2O$$

To determine whether MPO might oxidize HDL in vivo, we isolated HDL from plasma from subjects with established cardiovascular disease (CVD) and from age- and sex-matched healthy subjects [21]. HDL was delipidated and hydrolyzed, and the amino acid hydrolysate was analyzed by derivatization and negative-ion chemical ionization gas chromatography/MS. The level of protein-bound 3-chlorotyrosine was 13 times higher in circulating HDL from the patients with established CVD than in circulating HDL from the healthy subjects. These observations strongly support the hypothesis that HOCl derived from MPO contributes to HDL oxidation in vivo [21].

Using the same approach, we demonstrated that HDL isolated from subjects with CVD contains elevated levels of protein-bound 3-nitrotyrosine [22]. Moreover, levels of both 3-chlorotyrosine and 3-nitrotyrosine were higher in HDL isolated from atherosclerotic lesions than in plasma HDL. Investigators at the Cleveland Clinic Research Foundation have reported similar results [23].

Collectively, these observations suggest that elevated levels of 3-chlorotyrosine and 3-nitrotyrosine in circulating HDL might represent novel markers for clinically significant atherosclerosis. Demonstrating that apoA-I is chlorinated or nitrated on specific residues in vivo would clearly have important implications for understanding the role of oxidative events in the pathogenesis of vascular disease. Such observations would be of even greater interest if site-specific modifications of apoA-I were associated with alterations in the protein's biologic properties.

MPO-mediated Chlorination but not Nitration of ApoA-I Impairs Cholesterol Efflux Activity

We examined the effects of MPO-mediated oxidation of apoA-I on its ability to remove cellular cholesterol by the ABCA1 pathway [21,24]. Exposing apoA-I to MPO or its major product HOCl progressively and severely impaired the ability of apoA-I to remove cellular cholesterol from ABCA1-transfected cells. In contrast, exposing apoA-I to the MPO nitrating system had little effect on its cholesterol efflux activity, despite extensive nitration of Tyr192 [24]. These results show that modifying Tyr192 alone cannot account for the loss of function.

Our findings indicate that oxidation of apoA-I with HOCl dramatically reduces the apolipoprotein's ability to remove cholesterol from cells by the ABCA1 pathway. These observations strongly suggest that MPO-mediated oxidation of apoA-I in artery walls would severely impair one of its atheroprotective functions.

HOCl Chlorinates Specific Tyrosine Residues in the YxxK Motif of ApoA-I

To examine whether sequence-dependent chlorination of tyrosine residues occurs in biologically relevant proteins, we oxidized HDL and apoA-I with HOCl and looked for tyrosine chlorination products in apoA-I. ApoA-I contains seven tyrosine residues. Importantly, most of them are near the ends of amphipathic α-helices, which play critical roles in lipid binding and reverse cholesterol transport [1,2,25].

Using liquid chromatography-MS/MS, we demonstrated that Tyr192 was the major target of chlorination in apoA-I [24,26]. Importantly, this residue resides in a YxxK motif (Y, tyrosine; K, lysine; x, nonreactive amino acid).

MPO Impairs ABCA1-dependent Cholesterol Export from Cells by Oxidizing Methionine Residues and Promoting Site-specific Chlorination of Tyrosine 192 in ApoA-I

Studies with synthetic peptides indicated that lysine residues promote the region-specific chlorination of tyrosine residues via the formation of chloramines [26].

$$\text{Lysine-}NH_2 + \text{HOCl} \rightarrow \text{Lysine-NHCl} + H_2O$$
$$\text{Lysine-NHCl} + \text{Tyrosine} \rightarrow \text{3-Chlorotyrosine} + \text{Lysine-}NH_2$$

To test the proposal that lysine residues can direct tyrosine (Y) chlorination in proteins, we engineered a series of mutations in human apoA-I [27]. When the K residue in the YxxK motif next to Y192 was mutated to arginine, chlorination of Y192 by MPO was blocked. Y166 is resistant to chlorination and does not reside in a YxxK motif. When we introduced the YxxK motif into this region of apoA-I, we obtained a high yield of chlorinated Y166.

Importantly, we also found that methionine residues in apoA-I were oxidized quantitatively by MPO, and that this process was completely reversed by treatment with PilB, a methionine sulfoxide reductase [27]. Phenylalanine (F), in contrast to Tyr, is resistant to chlorination by MPO. Remarkably, when the Y192F mutant of apoA-I was exposed to MPO and then incubated with PilB, its ability to promote cholesterol efflux by the ABCA1 pathway was almost completely restored.

These observations strongly support the proposal that the YxxK motif directs the region-selective chlorination of Tyr residues in apoA-I [27]. They further suggest that a combination of Y192 chlorination and methionine oxidation is both necessary and sufficient for depriving apoA-I of its ABCA1-dependent cholesterol transport activity.

Clinical Implications

Collectively, these observations suggest that chlorinated and nitrated HDL could serve as markers for active cardiovascular disease in humans. They also suggest that HDL oxidation impairs the lipoprotein's ability to promote reverse cholesterol transport, raising the possibility that MPO provides a specific pathway for generating dysfunctional HDL [9,14,28]. Thus, MPO might be a potential therapeutic target for preventing cardiovascular disease in humans.

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