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Cholesterol Reduction and Macrophage Function: Role of Paraoxonases

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Abstract

Purpose of review—Unregulated uptake of oxidized LDL by macrophages to form foam cells is the hallmark for atherosclerosis. The paraoxonase (PON) family of enzymes play a critical role in attenuating atherosclerotic lesion formation by hydrolyzing lipid peroxides (LOOHs) and preventing the oxidation of LDL particles and by enhancing HDL-mediated cholesterol efflux. Findings in recent years suggest novel mechanisms by which PON isoforms interact with macrophages to regulate cholesterol metabolism and cellular function.

Recent findings—The association of PON with HDL particles facilitates binding of the particle to macrophages and ABCA1-dependent cholesterol efflux. The hydrolysis of membrane phospholipids by PON generates lysophosphatidylcholine which is shown to regulate expression of cholesterol transport proteins. The PON family also regulates multiple aspects of macrophage function. PON attenuates inflammation and prevents induction of apoptosis *via* activation of an SR-B1-dependent signaling mechanism. PON limits macrophage-dependent oxidant formation by preventing activation of the membrane-associated NADPH oxidase and by stabilizing mitochondria. PON also promotes the differentiation of macrophages to an anti-inflammatory phenotype. This function appears to be independent of PON enzymatic activity and, rather, is dependent on the ability of endogenous sulfhydryls to neutralize pro-inflammatory peroxides.

Summary—In recent years, the therapeutic efficacy of HDL-based therapies has been subject to dispute. Pharmacological approaches that target an increase in the expression and/or activity of PON may facilitate macrophage cholesterol metabolism and attenuate inflammatory injury.

Keywords

paraoxonase; atherosclerosis; macrophage; cholesterol; inflammation

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Introduction

The paraoxonases (PON) are a family of enzymes (PON1, PON2 and PON3) that are encoded by adjacent genes on human chromosome 7 (1–3). PON1 is synthesized and secreted by the liver and is associated with HDL particles (2). The PON2 isoform is expressed in hepatocytes, macrophages and other cell types where it is found in association with the endoplasmic reticulum and mitochondria (4,5). PON3 is similar to PON1 in that both are synthesized in the liver, are associated with HDL particles and possess strong anti-oxidant properties (6,7). PON isoforms share some common substrates but also display specific substrate specificities (1,2,8). PON1 possesses paraoxonase activity due to its ability to detoxify organophosphate compounds such as paraoxon (9,10). It also displays lactonase activity which facilitates the metabolism of drugs and endogenous heterocyclic esters and prevents the homocysteinylation of proteins. Importantly, PON1 possesses esterase activity that mediates the hydrolysis of oxidized lipids in lipoprotein particles and atherosclerotic lesions, thus inhibiting plaque progression (9). PON2 exerts prominent lactonase activity that has been shown to reduce quorum sensing, biofilm formation and thus bacterial virulence (11). In this manner, PON2 may play an important role in the host defense response. PON3 possesses paraoxonase and esterase activities, however, these are significantly reduced compared to PON1 (3,6,7). A more prominent role for PON3 in drug metabolism has been suggested (2). The specific physiological substrates for PONs have not been clearly identified but likely include oxidation products of arachidonic acid and docosahexaenoic acid (2).

The current understanding of PON function under normal and pathophysiological conditions is largely derived from studies of the PON1 isoform (12,13). While hyperlipidemia and inflammation play an important role in the regulation of PON1 expression and activity, it is also subject to epigenetic regulation (14). Several CpG sites on the PON1 gene are susceptible to methylation, and this modification is associated with a reduction in esterase activity (15). PON1 may also be attenuated by microRNA 616 (14,16,17). Post-translational modification of PON1 has been reported in the presence of high glucose. In this respect, the glycation of PON1 is associated with a reduction in enzymatic activity (18). In the context of inflammation, lipopolysaccharide (LPS) and cytokines reduce hepatic PON1 expression (19). Lipid lowering therapies may prevent or reverse this effect (20). Another study shows that atorvastatin upregulates PON2 expression in macrophages by a mechanism involving a reduction in cellular cholesterol content (21). The macrophage is thus an important site of PON action. The focus of this review is to summarize the role of HDL-associated and cellular PON isoforms in the regulation of macrophage cholesterol content and associated effects on macrophage phenotype and function.

HDL, PON1 and Reverse Cholesterol Transport

A major atheroprotective role of HDL is to mediate reverse cholesterol transport (RCT). This is achieved through the interaction of HDL particles with the ATP binding cassette transporters ABCA1 and ABCG1. The scavenger receptor class B type 1 (SR-B1) represents an alternate pathway for HDL-mediated cholesterol efflux. While PON1 protects HDL and LDL particles against oxidation, it also enhances RCT by facilitating the binding of HDL

particles to macrophages (22–24). Incubation of J774A.1 macrophages with fluorescently labeled PON1 shows that PON1 binds to low-affinity sites and is internalized (25). Further, macrophage cholesterol efflux was reduced by 35% when cells were exposed to HDL in the presence of an antibody to PON1. Similarly, co-incubation of HDL with the PON1 antibody reduced the ability of HDL to inhibit the macrophage-dependent oxidation of LDL (25).

Lysophosphatidylcholine (LysoPC) plays a role in HDL-PON1-induced cholesterol efflux (26). Incubation of oxLDL with PON1 has been shown to liberate LysoPC. Treatment of J774A.1 macrophages with the oxLDL-PON1 complex increased apoA-I-mediated cholesterol efflux compared to oxLDL in the absence of PON1 (26). Purified LysoPC mimicked effects of oxLDL-PON1 on macrophage cholesterol efflux. Additional studies revealed that oxLDL-PON1 increased expression of liver X receptor (LXR) and peroxisome proliferator-activated receptor γ (PPAR γ), known inducers of ABCA1 and ABCG1 (26). Inactivation of PON1 was associated with inhibition of macrophage ABCA1/ABCG1 expression. Finally, macrophages were loaded with radiolabeled cholesterol and incubated with oxLDL-PON1 prior to injection in wild-type mice. The fecal elimination of macrophage-derived cholesterol increased compared to that of mice treated with oxLDL in the absence of PON1 (26). It was concluded that PON1 increases cholesterol efflux in macrophages by hydrolyzing oxidized phospholipids, thus inducing LysoPC release and upregulation of ABCA1 and ABCG1 (26).

Incubation of mouse peritoneal macrophages (MPM) with HDL isolated from PON1 transgenic mice enhanced cholesterol efflux compared with HDL isolated from PON1^{-/-} mice (23). This response was associated with increased binding of the HDL-PON1 complex to the cell surface. Further, incubation of cells with an anti-SR-B1 antibody did not influence cholesterol efflux activity induced by the HDL-PON1 complex. Rather, cholesterol efflux was mediated by ABCA1 and required PON1 activity (23). HDL-PON1 binding to ABCA1 and the resulting cholesterol efflux were associated with an increase in the macrophage content of LysoPC (23). Thus, PON1 enhances HDL binding and cholesterol efflux *via* the enzymatic conversion of macrophage membrane phospholipids to LysoPC (23).

Fuhrman *et al* similarly reported that PON1 stimulates HDL binding and cholesterol efflux in MPMs (27). In this study, ABCA1 expression was similar in MPMs isolated from C57Bl/6 and PON1^{-/-} mice, however, deletion of PON1 significantly reduced SR-BI expression (27). The binding of HDL to MPMs from PON1^{-/-} mice was reduced compared to MPMs from wild-type mice, but this did not have a significant effect on cholesterol efflux (27). Expression of SR-BI was restored by treatment of MPMs with recombinant PON1 but not catalytically inactivated PON1. Addition of PON1 to MPMs induced the formation of LysoPC and upregulation of SR-B1 by a mechanism involving activation of extra-cellular signal-regulated kinase (ERK1/2) and phosphatidylinositol-3 kinase (PI3K) (27). Under these conditions, the interaction between PON1 and SR-B1 was required for protection against cell injury and apoptosis. These data provide strong evidence to show that PON1: 1) binds to specific sites on the macrophage cell surface; 2) facilitates HDL-mediated RCT; 3) regulates macrophage redox status; and 4) inhibits the induction of apoptosis (25).

Paraoxonase and oxidant stress

Both PON1 and PON3 are components of HDL that reduce the atherogenicity of lipoproteins by hydrolyzing oxidized lipids (28). Recombinant PON1 and PON3 reduce the oxidation of LDL *in vitro*, with PON1 showing a greater inhibitory effect (29). As a result, the uptake of atherogenic lipoproteins by macrophages is significantly reduced (28,29). In addition to degrading LOOHs, HDL-associated PON1 reduces oxidant formation in macrophages (27). The capacity of MPMs isolated from PON1^{-/-} mice to oxidize LDL is increased compared to wild-type MPMs and is associated with an increase in macrophage LOOH content (28). In contrast, macrophages derived from PON1 transgenic mice display a reduced LOOH content and limited capacity to induce LDL oxidation.

MPMs isolated from PON1^{-/-} mice on either a wild-type or apoE^{-/-} background display an increase in LOOH content and oxidized glutathione levels compared to corresponding controls (30). *In vitro* studies show that these cells produce superoxide anion which contributes to the oxidation of LDL. These responses are associated with translocation of p47^{phox} to the plasma membrane and activation of the pro-oxidant enzyme NADPH oxidase. Treatment of MPMs isolated from PON1^{-/-}/apoE^{-/-} mice with PON1 significantly reduced superoxide formation and LOOH content (30). Further, atherosclerotic lesions in PON1^{-/-}/apoE^{-/-} mice were significantly increased compared to apoE^{-/-} control mice. These results underscore the role of PON1 in the regulation of macrophage redox status and atherosclerotic lesion formation (30). Other data show that NADPH oxidase-dependent superoxide formation is a stimulus for PON2 expression in MPMs. Intraperitoneal administration of thioglycollate is associated with the activation of MPMs. It was shown that PON2 expression and activity were significantly reduced in MPMs isolated from p47^{phox}^{-/-} mice treated with thioglycollate (5). These data suggest that activation of NADPH oxidase is required for the upregulation of PON2 in macrophages.

PON2 improves mitochondrial function

Mitochondria are a source of free radicals that contribute to oxidative injury and atherogenesis (31,32). PON2 attenuates mitochondrial oxidant formation in macrophages and other cell types (4,33,34). PON2 is localized to the inner mitochondrial membrane in proximity with complex III of the electron transport chain. Deletion of PON2 in apoE^{-/-} mice increases oxidative stress and enhances atherosclerotic lesion formation (34). Analysis of mitochondria isolated from liver and MPMs demonstrated a reduction in the activities of mitochondrial complexes I and III, oxygen consumption and ATP formation (34). In contrast, expression of PON2 in apoE^{-/-} mice attenuated these changes in mitochondrial bioenergetics and reduced fatty lesion formation (34). It was further confirmed that PON2 reduces superoxide formation at complexes I and III and thus prevents the induction of apoptosis (33). These data suggest that PON2 improves mitochondrial function and reduces oxidative stress by increasing the activity of electron transport complexes (33,34).

Paraoxonase and inflammation

The presence of PON1 in HDL particles is thought to contribute to anti-inflammatory properties of the lipoprotein which enable the degradation of oxidized lipid species in LDL particles (35). PON1 inhibits the ability of minimally modified LDL or oxidized L- α -1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphorylcholine (oxPAPC) to induce monocyte binding and transmigration across endothelial cell monolayers (35). Under these conditions, mass spectral analysis revealed a decrease in the *m/z* ratio of oxPAPC fragments in samples treated with PON1 (35). Further, PON1 deletion induces a pro-inflammatory environment in mice that promotes leukocyte adhesion and thrombus formation (36). PON1 has been shown to directly impact inflammation at the level of the macrophage (37). Treatment of bone marrow-derived macrophages (BMDMs) isolated from C57Bl/6 mice with LPS and interferon γ (IFN γ) induces the secretion of tumor necrosis factor α (TNF α) and interleukin 6 (IL-6). LPS/IFN γ exposure of BMDMs from PON1 transgenic mice, however, attenuated cytokine release (37). In additional studies, cytokine secretion was monitored in J774.A1 macrophages treated with recombinant PON1 or catalytically-inactive PON1 mutants. Surprisingly, PON1 mutants were as effective as recombinant PON1 in inhibiting TNF α secretion. This result suggested that the anti-inflammatory response to PON1 was not dependent on its enzymatic activity (37). Rather, anti-inflammatory effects were shown to be dependent on SR-B1. In support of this, the PON1-mediated inhibition of cytokine secretion in BMDMs was attenuated by both an anti-SR-B1 antibody and siRNA directed against SR-B1. It was proposed that SR-B1 acts as a signaling receptor that modulates downstream signaling pathways that regulate cytokine release (37).

Paraoxonase, macrophage phenotype and resolution of inflammation

PON expression influences macrophage differentiation, phenotype and function. Two populations of activated macrophages have been identified that can be distinguished on the basis of their phenotypic differentiation, cell morphology and function (38). Pro-inflammatory M1 macrophages are induced by Th1 cytokines (IFN- γ , IL-2, TNF α) and LPS (39,40). Alternatively-activated M2 macrophages represent the second phenotype. M2 macrophages resolve inflammation and injury by suppressing cytokine secretion and by promoting wound healing and tissue remodeling (38–41). An analysis of MPMs isolated from PON1-treated C57Bl/6 mice reveals prominent differences in cell morphometry compared to treatment with saline (42). MPMs isolated from C57Bl/6 mice were large, granulated and showed a low ratio of nucleus to cytoplasm. In contrast, macrophages from PON1 treated mice were smaller, contained less cytoplasm and a low level of granulation (42). The integrin CD11b is expressed on the surface of monocytes and plays a role in cell adhesion and macrophage differentiation (43). An increase in CD11b and CD36 expression is associated with MPMs of PON1^{-/-} mice (42). Intraperitoneal administration of recombinant PON1 to PON1^{-/-} mice resulted in a greater reduction in CD11b and CD36 expression compared to C57Bl/6 mice. Expression of both cell markers was also reduced by treatment of THP-1 macrophages with PON1 (42). The inhibitory effect of PON1 on macrophage differentiation was not related to the catalytic activity of PON1 but rather to the presence of sulfhydryl groups on the enzyme (42). The authors suggested that PON1 can reduce the level of cellular oxidants *via* the sulfhydryl-mediated neutralization of cellular

peroxides which are known to induce macrophage differentiation (42). An increase in cellular oxidant formation by complex I and other respiratory proteins is thought to stimulate CD11b expression. In these studies, the complex I inhibitor rotenone was shown to inhibit CD11b expression. It was suggested that the PON1-mediated inhibition of CD11b is due to the ability of PON1 to interact with complex I and thereby reduce oxidative stress (42).

PON2 also regulates macrophage differentiation (44). MPMs isolated from PON2^{-/-} mice display a pro-inflammatory phenotype (increased TNF α , IL-6 and arginase II) compared to wild-type mice (44). Further, the expression of IL-10 and arginase I, markers of anti-inflammatory M2 macrophages, was significantly reduced in MPMs of PON2^{-/-} mice compared to controls. IL-4, an activator of M2 macrophage differentiation, did not alter the pro-inflammatory phenotype of MPMs from PON2^{-/-} mice (44). These cells were characterized by enhanced phagocytic activity and oxidant formation. Transfection of human PON2 into macrophages from PON2^{-/-} mice inhibited the induction of a pro-inflammatory M1 phenotype and promoted M2 phenotypic differentiation. These data suggest that PON2 inhibits the differentiation of monocytes to a pro-inflammatory phenotype and can act as a switch from an M1 to an M2 phenotype in macrophages (44).

Conclusions

Atherosclerotic lesion formation is associated with the biochemical modification of apolipoprotein B-containing lipoproteins. These particles are taken up by macrophages in the artery wall resulting in the formation of foam cells, which contribute to the development of atheroma. The major anti-atherogenic function of HDL is to mediate RCT. Since HDL becomes dysfunctional in some disease states, therapeutic approaches that increase the functional properties of HDL are needed (45). An important goal of drug design may be to develop formulations that increase the expression and/or activity of PON. As noted above, HDL-associated PON1 and PON3 play an important anti-atherogenic role by hydrolyzing LOOHs and preventing the oxidation of HDL and LDL particles. PON1 levels in HDL are higher than those for PON3, thus PON1 activity is predominant in the HDL particle. The principal function of PON2 is to limit oxidant stress at the cellular level. The PON family of enzymes also regulates macrophage function at multiple levels, as described below.

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Key points

- PON1 enhances RCT by facilitating the binding of HDL to macrophages. This requires LysoPC formation and upregulation of the cholesterol transporters ABCA1 and ABCG1.
- PON1 and PON2 reduce oxidant formation in macrophages by multiple mechanisms. First, PON1 prevents translocation of p47^{phox} to the cell membrane, thus preventing assembly of the NADPH oxidase complex. Second, PON1 and PON2 reduce superoxide formation by stabilizing mitochondrial respiration at the level of complexes I and/or III.
- PON1 inhibits cytokine release from macrophages by enzymatic and non-enzymatic mechanisms. The latter response is linked to an interaction between PON1 and SR-B1.
- PON1 and PON2 contribute to the resolution of inflammation by promoting the differentiation of macrophages to an anti-inflammatory phenotype. This function is likely independent of PON enzymatic activity and, rather, relies on the ability of endogenous sulfhydryls to neutralize pro-inflammatory peroxides.