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Invited critical review

Paraoxonase 1 and HDL maturation

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Understanding the kinetics and function of paraoxonase 1 (PON1) is becoming an important issue in atherosclerosis. Low PON1 activity has been consistently linked with an increased risk of major cardiovascular events in the setting of secondary prevention of coronary artery disease. Recent studies have shown that there is a specific interaction of myeloperoxidase (MPO)–apoAI–PON1 on HDL surface that seems to be germane to atherogenesis. MPO specifically inhibits PON1 and PON1 mitigates MPO effects. Surprisingly, very little is known about the routes by which PON1 gets integrated into HDL or its fate during HDL remodeling in the intravascular space. We have developed a method that assesses PON1 activity in the individual HDL subclasses with the aid of which we have shown that PON1 is present across the HDL particle range and preferentially in HDL_3, confirming data from ultracentrifugation (UC) studies. Upon HDL maturation ex vivo PON1 is activated and it shows a flux to both smaller and larger HDL particles as well as to VLDL and sdLDL. At the same time apoE, AI and AII are shifted across particle sizes. PON1 activation and flux across HDL particles are blocked by CETP and LCAT inhibitors. In a group of particles with such a complex biology as HDL, knowledge of the interaction between apo-lipoproteins, lipids and enzymes is key for an increased understanding of the yet multiple unknown features of its function. Solving the HDL paradox will necessitate the development of techniques to explore HDL function that are practical and well adapted to clinical studies and eventually become useful in patient monitoring. The confluence of proteomic, functional studies, HDL subclasses, PON1 assays and zymogram will yield data to draw a more elaborate and comprehensive picture of the function of HDL. It must be noted that all these studies are static and conducted in the fasting state. The crucial phase will be achieved when human kinetic studies (both in the fasting and post-prandial states) on HDL–PON1, apoA-I and lipid fate in the circulation are carried out.

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Abbreviations: apoA-I, apolipoprotein A-I; CAD, coronary heart disease; CETP, cholesteryl ester transfer protein; CVD, cardiovascular disease; DNTB, dinitrothiocyanobenzene; HDL, high density lipoprotein; HDL-C, high density lipoprotein cholesterol; LCAT, lecithin: cholesterol acyltransferase; LDL, low density lipoprotein; LDL-C, low density lipoprotein cholesterol; MPO, myeloperoxidase; NMR, nuclear magnetic resonance; PLTP, phospholipid cholesteryl ester transfer protein; PON1, paraoxonase 1; SAA, serum amyloid A; sdLDL, small-dense low density lipoprotein; TG, triglycerides; UC, ultracentrifugation; VLDL, very low density lipoprotein.

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1. Introduction

The high density lipoprotein (HDL) field is in a present-day paradoxical situation [1–7]. Notwithstanding abundant epidemiological data associating high HDL cholesterol (HDL-C) concentrations with reduced cardiovascular disease (CVD) risks, latest therapeutic efforts to increase HDL-C have thus far failed to show clinical benefits while Mendelian genetic studies indicate that genetic variants that control HDL-C are not causally linked to CVD [8]. This paradox may well be due to the nature of the information provided by HDL-C. As a snapshot of the steady-state cholesterol pool, HDL-cholesterol levels provide no direct evidence on the rate of cholesterol-flux from vascular macrophages to the liver, which is influenced by many factors beyond the mass of HDL cholesterol alone. Moreover, circulating HDL-cholesterol concentrations provide no information regarding the anti-inflammatory, antioxidant, antithrombotic, and endothelial function-promoting activities of HDL [4]. On the other hand, overwhelming animal research directly shows that apoA-I has anti-atherogenic functions. This seeming contradiction highlights the need for a better understanding of HDL and its components. HDL is indeed a general term for a highly heterogeneous group of particles containing multiple proteins, including apolipoprotein A-I (apoA-I), the major structural protein of HDL, and additional proteins involved in hemostasis, thrombosis, immune and complement systems, growth factors, receptors, and hormone-associated proteins [5,6,9–16]. The current view maintains that distinct HDL particle subpopulations composed of unique clusters of specific HDL associated proteins perform specific biological functions. HDL particles containing paraoxonase 1 (PON1), an athero-protective protein, show improved anti-oxidative, anti-inflammatory and lipid cargo carrying functions.

In a group of particles with such a complex biology as HDL, knowledge of the interaction between apo-lipoproteins, lipids and enzymes is instrumental for the understanding of multiple unknown features of its function. Solving the HDL paradox will necessitate the development of techniques to explore HDL function that are practical and well adapted to clinical studies and eventually become useful in patient monitoring. In this review we will focus on one functional protein in HDL, paraoxonase 1 (PON1), specifically on the new developments about its interaction with HDL during its maturation. The reader is referred to current excellent reviews on other aspects of PON1 biology [17–27].

2. Paraoxonase 1 (PON1) and its interaction with HDL

PON1 is a circulating esterase and lactonase mostly carried on HDL [16,18,27]. Most PON1 circulates associated with HDL, however small amounts are found in very low density lipoprotein (VLDL) [28] and in chylomicrons [29]. It has been suggested that PON1 may use VLDL as a vehicle to get into HDL [28].

PON1 is secreted by the liver, it needs apoA-I for full activation and it is associated with apoJ. Surprisingly, very little is known about the routes by which it gets integrated into HDL or its fate during HDL remodeling in the intravascular space [16,18,30]. The crystal structure of PON1 implies that PON1 could be an interfacially activated, flexible enzyme. HDL particles carrying apoA-I bind PON1 with high affinity and thus stabilize the enzyme more than 100-fold while stimulating its lipolactonase activity (≤20-fold relative to the delipidated form) [31–34]. Structural studies and models indicate that HDL anchoring is mediated by the N-terminal helix of PON1 (named H1) and another amphipathic helix present in the active site (named H2). Segments beyond H1 are also involved in HDL binding [31–34].

While its endogenous substrates are unclear, it is believed that they include oxidized lipids because PON1 shows anti-oxidant activities. PON1 also stimulates HDL mediated endothelial nitric oxide synthase (eNOS)-dependent NO production and enhances cholesterol efflux from macrophages [18,19,22,25–27,35–39]. Several epidemiological and cohort studies have provided persuasive arguments for a role for PON1 in atheroprotection, through its ability to prevent lipid oxidation and limit atherosclerotic lesion development [18,19,22,25–27,35–39]. Animal studies reinforce this argument and provide mechanistic explanations. For instance, PON1-knockout mice have accelerated atherosclerosis. Conversely, PON1-transgenic mice are protected from atherosclerosis and oxidative stress [40,41]. PON1 hydrolyzes lipoprotein-associated peroxides and lactones. Development of strategies to increase PON1 activity in vivo would be a fundamental achievement. PON1 is indeed sensitive to its milieu. In human carotid lesions it interacts with its components altering them and is in turn affected by them [19,42–44]. Raised PON1 expression and activity in response to nutritional factors occur in animal model and in humans. Oleic acid increases PON1 activity in mice and humans and so does a Mediterranean-type meal [45,46]. Rats fed quercetin showed increase in PON1 gene expression and in serum and liver PON1 activity, respectively [19]. Mice fed with red wine or its polyphenol quercetin showed significant induction of hepatic PON1 mRNA levels and pomegranate juice or its phenolics (punicalagin, gallic acid, ellagic acid) show similar effects [43,47,48]. We have shown comparable effects with *Ilex paraguariensis*, a very popular beverage in South America with high content of chlorogenic acids and rutin [49–52]. Phospholipid derivatives also have the ability to affect PON1 activities. Phosphatidylcholine with unsaturated fatty acids strikingly increases free PON1arylesterase activity. HDL isolated from serum enriched with di-oleoylphosphatidylcholine considerably enhanced HDL PON1 activities in comparison to control HDL [19,42]. As we discuss later, it is likely that the activation of PON1 that happens during HDL maturation is in part due to its interaction with diverse phospholipid moieties.

PON1 may be in equilibrium between lipoprotein-bound and free forms. PON1 dissociates from HDL in physiological conditions and increased free PON1 has been associated with diseases with high oxidative stress [43,53]. Serum PON1 can be inactivated in vitro with chemicals, and the slopes of inactivation assay depend on its association or not with lipoproteins. Inactivation occurs in 2 phases [31]. The two inactivation phases seem to belong to two PON1 populations. This provides an insightful analytical tool: the slow phase parallels PON1 tightly bound to HDL-apoA-I, and the fast phase corresponds to “free” PON1. The fast-inactivating fraction is consequently more likely to match up to PON1 bound to different HDL subclasses, and/or other lipoprotein particles. The differences in phases of the inactivation assay differ with PON1 variants, including the 192R/Q polymorphism (the most prevalent). In the RR genotype sera, PON1 is tightly associated with HDL-apoA-I, whereas QQ and RQ sera show important fractions of “free” PON1 (18–46%). Further research is needed to elucidate the precise nature of the two PON1 populations.

In clinical studies, diabetic patients show increased free PON1 associated with lower total PON1 activities [31]. PON1 binding to HDL can be modulated by diet as well [43]. Bound PON1 was significantly increased by 32% following pomegranate juice (PJ) consumption, suggesting that PJ consumption resulted in increased free PON1 binding to the HDL [43]. We have also shown and will discuss later that PON1 gets activated ex vivo and this may also reflect shifts from “loosely” to “tightly” bound PON1 [54].

3. HDL subclasses: maturation

Notwithstanding the previous discussion on free PON1, it is a fact that over 80% of PON1 circulates bound to HDL [5,11,19,55]. However, as indicated earlier HDL is a very broad term that encompasses a heterogeneous group of lipoproteins that may be classified by increasing size in HDL_3c, HDL_3b, HDL_3a, HDL_2b, and HDL_2a, as measured by native PAGE [11,14,55,56]. HDL subclasses are currently assessed and have been classified by other approaches such as gradient gel electrophoresis, vertical auto profile ultracentrifugation, nuclear magnetic resonance

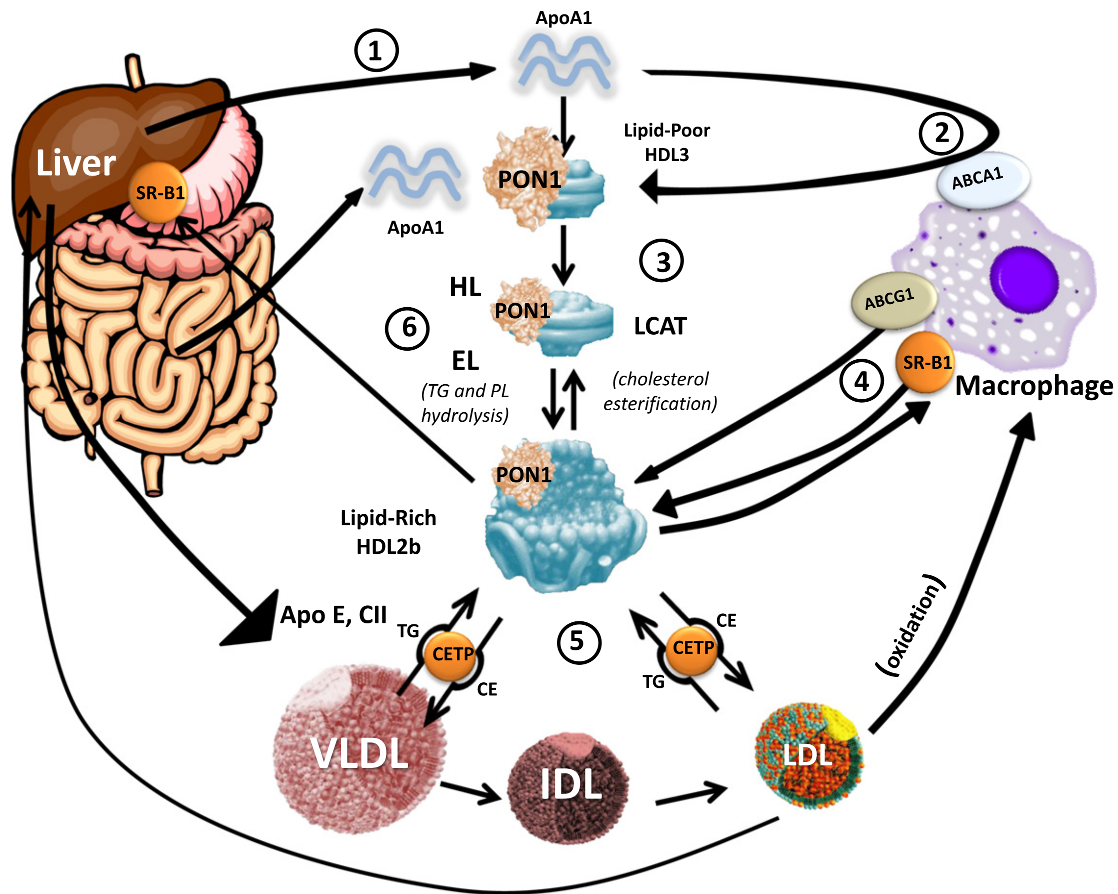

Fig. 1. HDL maturation and reverse cholesterol transport. 1. apoA-I is synthesized by the liver (and intestine). 2. Macrophage ATP-binding cassette transporter A1 (ABCA1) transfers cholesterol and phospholipids onto lipid-poor apoA-I, forming prebeta HDL. 3. Lecithin–cholesterol acyltransferase (LCAT) stimulates HDL maturation by converting free cholesterol into hydrophobic cholesteryl-esters that are sequestered into the core of the HDL particle which becomes HDL_3 and then HDL_2 as it enlarges and matures. 4. HDL_2 mobilizes more cholesterol from macrophages and other cells through scavenger-receptor class B, type 1 (SR-BI), ATP-binding cassette transporter G1 (ABCG1), and other receptor-independent pathways. SR-BI binds bigger HDL particles and forms a complex, which allows cholesterol efflux onto HDL. SR-BI facilitates bi-directional cholesterol flux and permits HDL-cholesterol to ingress cells. ABCG1 is an intracellular cholesterol transporter which rearranges plasma membrane cholesterol molecules and in so doing facilitates its absorption by cholesterol acceptors. 5. Mature HDL_2 is a substrate for CETP, a lipid-transfer protein that transports cholesteryl-esters and triglycerides between HDL, VLDL, and LDL. Phospholipid transfer protein (PLTP, not shown) transfers phospholipids between VLDL and HDL. 6. Two lipases, endothelial lipase (EL) and hepatic lipase (HL) are important in HDL metabolism. EL has high phospholipase A1 activity and remodels HDL into small particles whereas HL is more effective for TG hydrolysis.

spectroscopy, and ion mobility [11,57–60]. These methods distinguish HDL particles of different sizes or densities that might have different atheroprotective properties [3,5,11,14,55,56,61–66]. However, the most sophisticated methods available today offer information on particle size and number or protein content or lipid content but none informs on any functional property of HDL [5,11,14,55]. As depicted in Fig. 1 apoA-I is mainly synthesized by the liver (and intestine). In the first step of HDL metabolism, macrophage ATP-binding cassette transporter A1 (ABCA1) transfers cholesterol and phospholipids onto lipid-poor apoA-I, forming pre-beta HDL [2]. Lecithin–cholesterol acyltransferase (LCAT) binds to HDL and stimulates its maturation by converting free cholesterol into hydrophobic cholesteryl-esters that are sequestered into the core of the HDL particle which becomes HDL_3 and then HDL_2. As it enlarges and matures HDL_2 mobilizes more cholesterol from macrophages and other cells through scavenger-receptor class B, type 1 (SR-BI), ATP-binding cassette transporter G1 (ABCG1), and other receptor-independent pathways [2]. SR-BI binds bigger HDL particles and forms a complex that allows cholesterol efflux onto HDL. SR-BI facilitates bi-directional cholesterol flux and allows HDL-cholesterol ingress to cells. ABCG1 is an intracellular cholesterol transporter which rearranges plasma membrane cholesterol molecules and in so doing facilitates its absorption by cholesterol acceptors [4].

Mature HDL_2 is a substrate for CETP, a lipid-transfer protein that shuttles cholesteryl-esters and triglycerides between HDL, VLDL, and LDL. Phospholipid transfer protein (PLTP, not shown in the figure) transfers phospholipids between VLDL and HDL. Two lipases, endothelial lipase (EL) and hepatic lipase (HL) are important in HDL metabolism [4]. EL has high phospholipase A-I activity and remodels HDL into small particles, whereas HL is more effective for TG hydrolysis. It similarly effects remodeling of HDL into smaller particles, but it stimulates the release of lipid-poor apoA-I [2,4]. Proteins associated with inflammation such as serum amyloid alpha (SAA) are known to effect remodeling of HDL, by releasing apoA-I [67].

Several studies have shown that discrete subclasses of HDL bear different patterns of proteins [6,68]. PON1 in plasma is present on a subset of approximately 1 of 8–10 HDL particles [2,3].

Which HDL fraction confers better cardiovascular protection is the question which still remains debatable. It has been suggested that the large HDL fraction is the most athero-protective, because CAD patients have lower levels of these particles than controls, and premenopausal women have more, as assessed by selective precipitation or NMR [2,5,11]. In contrast, small HDL particles are the best acceptors of cholesterol from peripheral tissues and also have better antioxidant properties than large HDL. Moreover, thiazolidinediones as well as fibrates, both drugs that increase HDL-cholesterol plasma levels, shift HDL size distribution towards small HDL particles [2,5,11]. Subjects with severe hypo-alphalipoproteinemia who do not develop CAD have a high proportion of small HDL suggesting an

atheroprotective role of these particles [11,55,69]. Small HDL particles are protein rich and lipid poor, as opposed to large particles, therefore the relative proportion of HDL subclasses is dependent on the parameter that is quantified (lipid, protein, diameter, number of particles) [55,62–65]. The wide diversity of methods used for measuring HDL subclasses is partly responsible for the seeming disagreement concerning which is the most anti-atherogenic fraction of HDL [3,5,11,14,55,56,61–66]. Where PON1 location is and how does it transfer between these HDL subclasses are important questions which, when answered, will bring some light on the controversy of which HDL subclass is more atheroprotective.

4. PON1 in HDL subclasses: studies on native lipoproteins

Which HDL subclasses contain PON1? Are the PON1-containing particles more protective? Is PON1 evenly distributed across the whole spectrum of HDL sizes? Notably, a recent study shows that HDL isolated from patients with CAD has compromised antioxidant and endothelial protective activities. This is associated with decreased PON1 activity in small HDL particles due to their modification by malondialdehyde [70–72]. PON1 protective role on the endothelium has been substantiated in many studies [39,64,70–75].

Former studies employing ultracentrifugation have suggested that PON1 activity resides preferentially in the smaller HDL_3 particles [11,14,55,62,64,65]. More recent proteomic studies have confirmed this finding and added the evidence that structural, and more importantly, functional proteins in HDL tend to cluster in particles with different functional properties [6,68]. HDL_3 is clearly a more potent antioxidant, in part due to its PON1 content.

To make some inroads into studying active PON1 distribution across HDL subclasses we developed a zymogram method that combines native gradient gel electrophoresis and PON1 activity measurement in the same gel [76]. The method avoids the harsh treatment of the sample with g forces and very high ionic strength inherent to ultracentrifugation. It allows for simultaneous analysis of HDL particle size distribution in patients' sera and of the PON-1 enzymatic antioxidant activity in each fraction. It has the potential of giving information on the differential protective role of HDL particles of different sizes and its putative predictive value. At present only a few reports have been published on this issue, employing lengthy ultracentrifugation protocols to separate 2–3 HDL particle sizes [11,14,55,56,64,65]. These procedures are not exempt of methodological bias due to the effects of the shear g forces and ionic strength on the delicate HDL particles. In our method, native lipoproteins are separated in a gel and activity is detected in situ, as depicted in Fig. 2. By this procedure, we perform densitometric analyses for the determination of PON1 activity in HDL subclasses that requires a simple protocol that can be applied to multiple samples simultaneously, and requires a few μl of serum. After scanning for PON1, gels are used for protein or lipid detection for a more global profile. Furthermore, the method allows for dry transfer and sequential native immunoblotting to characterize apolipoprotein distribution in HDL fractions in the same gel, which can be correlated to PON1 activity profiles [76]. As we show in Fig. 3, it permits the discovery of associations of PON1 activity not only in differently sized HDL particles but also in classes of particles, such as apoA-I vs apoA-I:A-II as well as potentially in apoE rich or poor HDL, or apoC-III HDL, which have attracted attention recently as another pro-atherogenic and pro-inflammatory set of particles. As shown in Fig. 2, with this method we provide evidence that at the same HDL-C level, healthy subjects display a large difference in the ratio of PON1

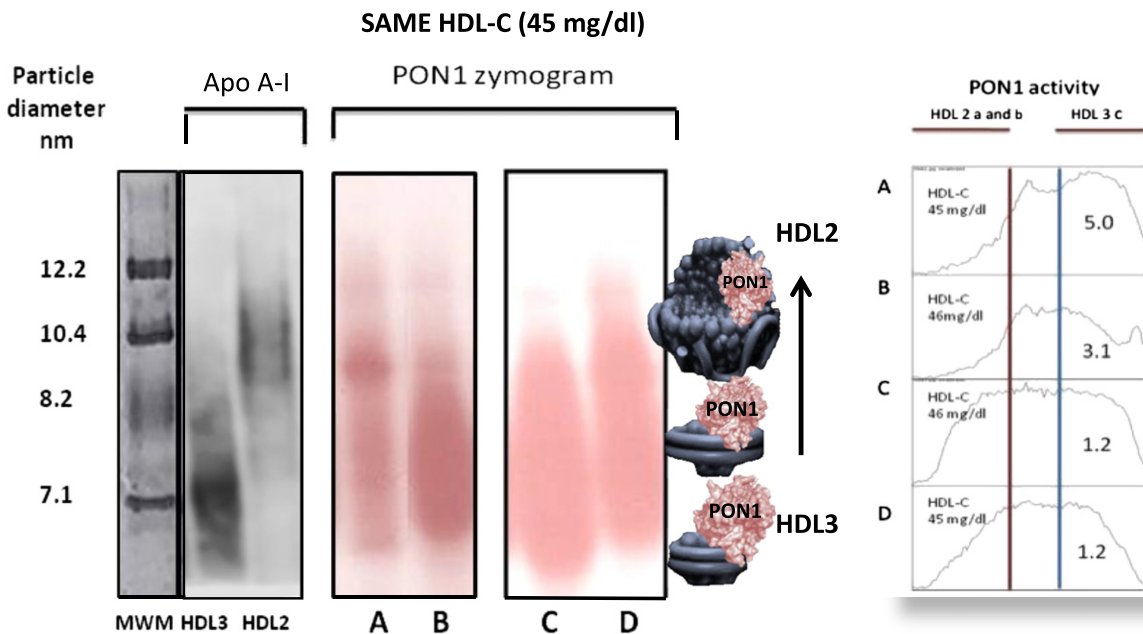

Fig. 2. Optimized zymogram method to study PON1 activity in native HDL subclasses. In this figure we show some of the data in [76]. On the left column, we show apoA-I distribution in HDL_2 and HDL_3 separated by ultracentrifugation. A, B, C, and D are four typical subjects from that study (out of 40) with the same HDL-C for whom PON1 activity was detected after electrophoresis with the method in [76]. Densitometries of those lanes are shown on the right. Note the wide differences found in PON1 activity and distribution even within the same HDL-C, suggesting potential new discriminant power for this assay.

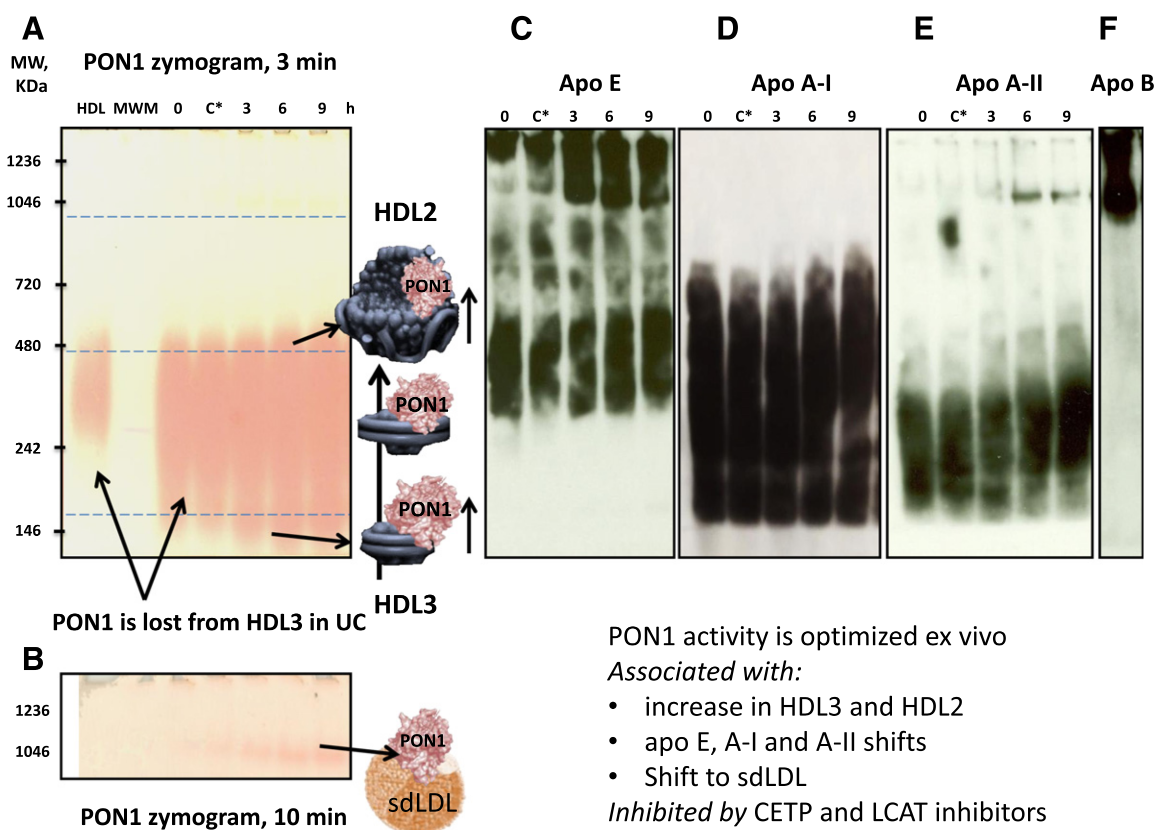

Fig. 3. Short-term ex vivo incubation of serum leads to PON1 activation and shift among HDL subclasses. This figure summarizes data from [79] where pooled sera from healthy volunteers were incubated ex vivo and PON1 activity, PON1 zymogram and apolipoprotein profiles were monitored. A and B show a zymogram of an ex vivo incubation experiment. C, D, E and F show sequential western blots for detection and co-localization of the indicated apolipoproteins. Compare PON1 distribution in purified HDL (UC) vis-à-vis total serum. Ultracentrifugation results in loss of PON1, preferentially from HDL_3. 0, 3, 6, and 9 stand for time of incubation at 37 °C in hours. C* is the control of incubation at 4 °C during the 9 h. Note the quick activation of PON1 activity associated with its increase in both very small HDL_3 and large HDL_2. In B, the upper part of the gel is shown after a longer time of incubation with PON1 substrate to enhance what is already apparent in A. PON1 shifts to small-dense LDL particles during HDL maturation. Western blots in C–F show concomitant shifts in apoE, A-I and A-II as well as confirm location of apoB-containing lipoproteins. Note in C and D that HDL_3 contains little apoE. The activation and shifts were inhibited by CETP and LCAT inhibitors.

activity in small vs. large HDL [76]. Since PON1 activity is larger in HDL_3 we have proposed that this difference has a potent predictive value for clinical risk assessment and therapeutic choice, an issue we begin to explore. In this regard we seek to further dissect the cause of a differential effect of PON-1 activity in HDL subclasses and what happens during HDL maturation.

5. PON1 shifts during HDL maturation

HDL starts its life as lipid-poor pre-beta HDL, which seems to lack PON1 [4,14,55]. Little is known about the dynamics of PON1 fate in HDL and especially the flux during maturation and remodeling. Understanding how and when PON1 gets integrated to the particle becomes an important research question. Factors that affect the remodeling and destiny of HDL apolipoproteins may contribute to vascular disease. PON1 activity depends on optimal association with HDL discrete subclasses and apolipoproteins; it is a cardioprotective factor and some drugs precisely affect the distribution of HDL subclasses. A better understanding of PON1 fate during remodeling may inform about alleged beneficial or deleterious effects of drugs as well as on pathogenic mechanisms for other conditions such as diabetes and metabolic syndrome. Ex vivo remodeling of HDL had been formerly employed to unravel some of the steps in this complex pathway [77,78]. It offers a view of the process catalyzed by LCAT, and lipid transfer proteins CETP and PLTP. One of the limitations of previous work on remodeling is that HDL was re-isolated from plasma by ultracentrifugation at different time points during the ex-vivo experiments, which disrupts the structure of HDL [77,78]. Our PON1 zymogram method allows for localization of active PON1 in native HDL subclasses not subjected to strenuous forces. As a first approach to understanding PON1 fate during short-term remodeling of HDL, we employed ex vivo incubation of human serum monitoring lactonase, arylesterase activities, analysis of PON1 distribution across HDL subclasses, and its association with apolipoprotein patterns [79]. This experimental design mimics the exchange between lipoproteins that takes place in the circulation in the absence of the lipases and receptors. The evidence provided could serve as a starting point to unravel these aspects of the very multifaceted and intricate HDL biology [79].

As shown in Fig. 3, we demonstrated a substantial activation (up to 20%) of both the lactonase and arylesterase activities of PON1 that is already apparent at 3 h ex vivo incubation of serum at 37 °C. An optimal PON1 environment appears to be generated in a short time of interaction between HDL and apoB-containing particles. Given the short time in which this activation of PON1 occurs, we posit that we may be in the presence of a physiological mechanism. Note the substantial loss of peripheral proteins, notably active PON1 when UC-purified HDL is compared with active PON1 in whole serum (Fig. 3). This clearly adds to the validity of this method to ascertain the distribution of active PON1 in HDL subclasses in both static and maturation studies. HDL is constantly subjected to remodeling by various elements including CETP and LCAT, lipases and receptors. Remodeling modifies the core and shell of HDL, respectively [79].

Along with the activation of PON1 ex vivo, we evidenced a simultaneous rapid redistribution of PON1 activity towards small HDL_{3c} and large HDL_{2b} (Fig. 3A). Additionally, together with these modifications, small-dense low density lipoprotein (sdLDL) acquires PON1 activity in

a parallel incremental time course (Fig. 3B). Small-dense LDL PON1 increases from 0 to 2% of the total PON1 value. Our work also suggests that lipid exchanges that ensue during HDL maturation ex vivo increase either PON1 content or PON1 activities (or both) in very small and very large HDL subclasses. A rise in HDL_3c PON1 activity that happens so quickly in vivo lends support to current understanding of these particles as the major antioxidant HDL subclasses. The increase in PON1 activity in large HDL_2 would be due to the maturation of smaller HDL_2, whereas the increase of PON1 in HDL_3c could be explained by maturation of discoidal, small prebeta HDL into HDL_3c. It should be noted that concentrations of several peptides of HDL are 2–3 orders of magnitude lower than apoA-I, HDL-C or HDL particles [6,11,68]. Accordingly, these microcomponents are not randomly distributed across HDL subclasses and this heterogeneity is overlooked by HDL-C, apoA-I or HDL subclass assays. As depicted in Fig. 3, our work suggests that PON1 swiftly shifts between HDL species as they mature, gets activated, interacts with sdLDL particles and gets transferred to them as well [79].

These PON1 shifts have an apolipoprotein correlate (Fig. 3, C–F). During the first phase of PON1 activation and shifts among particles, a parallel transfer of apoE ensues (Fig. 3C). ApoE initially increases in sdLDL and after 9 h it is lost from HDL and sdLDL but remains in VLDL. ApoA-I shifts towards larger particles (Fig. 3D) which becomes more apparent after 9 h and parallels the change in PON1. As HDL matures there is also a progressive shift of apoA-II towards larger HDL [79]. The above is consistent with the known maturation pathway for HDL. ApoA-II also transfers to sdLDL in parallel with PON1 and apoE (Fig. 3E). These are novel findings that may have physiological importance since these proteins are the ones shifted in HDL particles in diabetic patients [79]. Indeed, a recent proteomic study revealed that the main differences in HDL between type 2 diabetic young males and controls were found in PON1 and apoE distribution and this was associated with vascular stiffness [80].

Torcetrapib, which is a CETP inhibitor, and the LCAT inhibitor dinitrothiocyanobenzene (DTNB) prevented the activation of PON1 and enhanced its inactivation in longer incubations. Impaired activation of PON1 caused by CETP or LCAT inhibitors was associated with tardy shifts of apolipoprotein and a blunted transfer of PON1 from HDL to sdLDL. We conclude that HDL maturation optimizes PON1 activity and our data support the argument that PON1 activation is contingent on HDL remodeling via lipid exchange between HDL and apoB-containing lipoproteins [54]. These findings offer a likely mechanism to explain the failure of torcetrapib in clinical trials, which in spite of robust increases in HDL-C, led to an actual increase in CV episodes and death [81]. We show that torcetrapib reduces PON1 activation by limiting its exchange among lipoproteins and by that token inhibits PON1 activity in a time dependent manner [54]. This harmful effect of CETP inhibitors on a cardio-protective function of HDL warrants further exploration.

6. Interaction of PON-1 and myeloperoxidase: the missing link between inflammation and atherosclerosis that may help explain the HDL paradox?

Myeloperoxidase (MPO) selectively induces HDL oxidation within the artery wall converting an antiatherogenic lipoprotein into potential atherogenic forms [82–86]. MPO is a source of reactive oxygen species during inflammation and oxidizes apolipoprotein A-I (apoA-I) of HDL, impairing

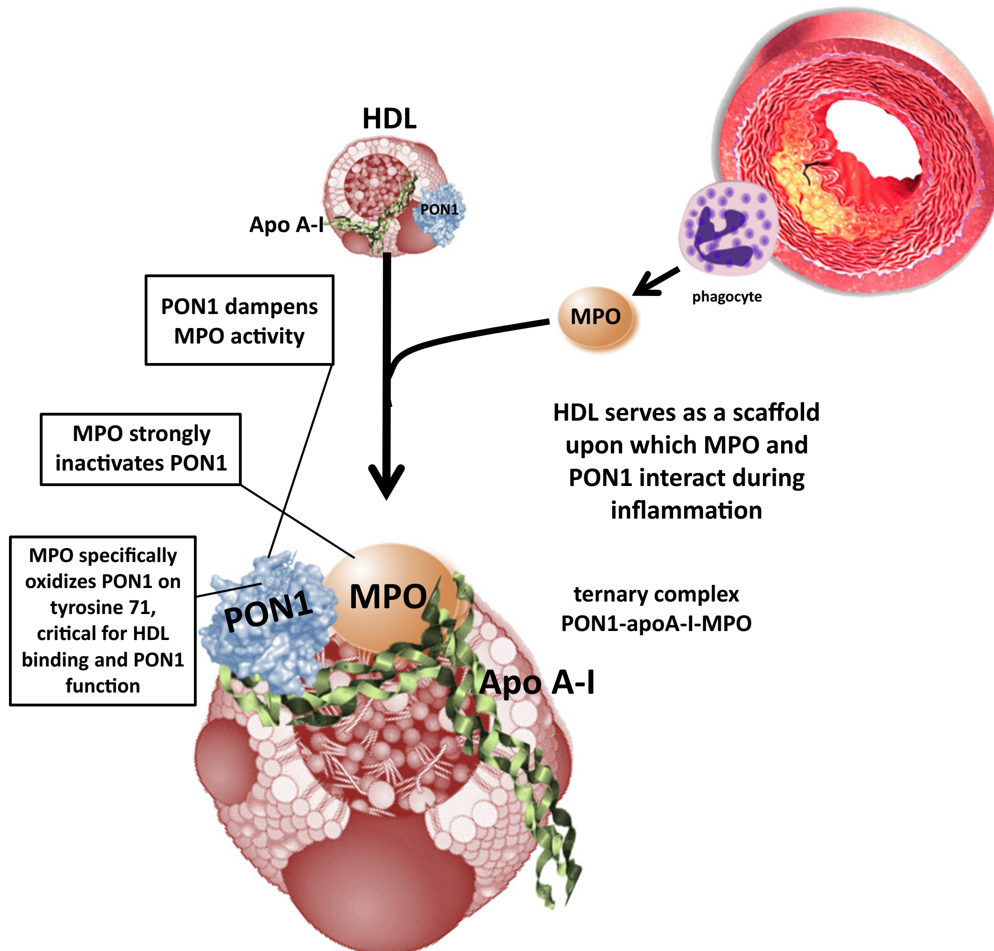

Fig. 4. HDL as a scaffold for MPO–PON1–apoA-I interactions. MPO, PON1, and HDL bind to one another, establishing a ternary complex, where PON1 moderately hinders MPO activity, while MPO inactivates PON1. MPO specifically oxidizes PON1 on tyrosine 71 (Tyr71). This reaction appears to be clinically relevant.

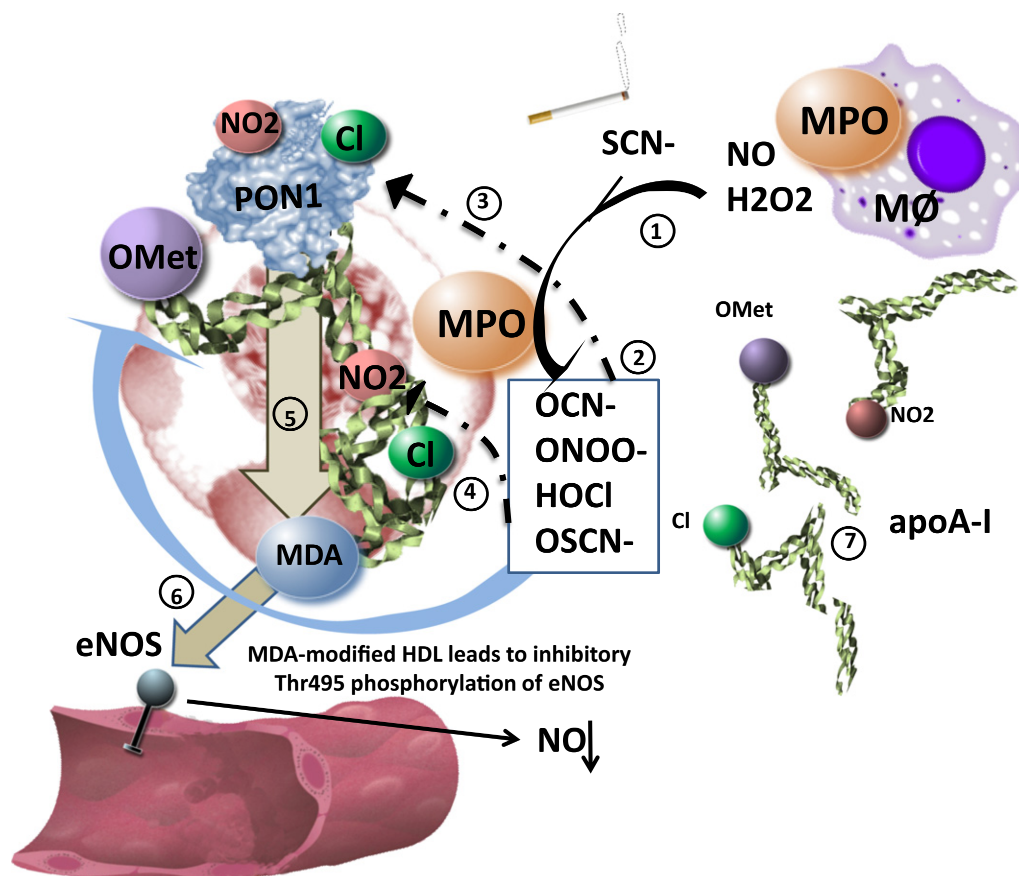

Fig. 5. Actions of free radicals on PON1, apoA-I and nitric oxide metabolism. 1. Macrophages and other phagocytes secrete MPO, H_2O_2 and NO, and thiocyanates may come from pollutants. 2. A series of reactions primed by MPO produces peroxynitrite, HOCl and other radicals that attack PON1 (3) and apoA-I (4) in HDL. HDL with modified PON1 is dysfunctional and malondialdehyde (MDA) adducts accumulate in apoA-I (5). HDL thus modified sets a series of reactions that result in phosphorylation of eNOS and reduction of endothelial NO, leading to vasoconstriction and ischemia. Free forms of modified apoA-I are found in the artery wall in coronary arteries from patients (7).

its athero-protective functions. Moreover, as depicted in Fig. 4, MPO, PON1, and HDL bind to one another, establishing a ternary complex, where PON1 moderately hinders MPO activity, while MPO inactivates PON1 [84]. MPO specifically oxidizes PON1 on tyrosine 71 (Tyr71). This reaction appears to be clinically relevant. Indeed, this is a modified residue found in human atheroma that is critical for HDL binding and PON1 function [82,84]. HDL thus serves as a scaffold, upon which MPO and PON1 interact during inflammation, whereupon PON1 binding partially inhibits MPO activity, and MPO promotes site-specific oxidative modification and impairment of PON1 and ApoA-I function.

As depicted in Fig. 5, PON1 is highly susceptible to free radical attack and its protective role can sometimes be overwhelmed when the flux of these compounds is too large, such as in an inflamed atheroma plaque as we and others have previously shown [6,51,87]. Macrophages and other phagocytes secrete MPO, H_2O_2 and NO, and thiocyanates may come from pollutants. A series of reactions primed by MPO produces peroxynitrite, HOCl and other radicals that attack PON1 as well as apoA-I in HDL [88]. HDL with modified PON1 is dysfunctional and malondialdehyde (MDA) adducts accumulate in apoA-I HDL thus induces a series of reactions that result in phosphorylation of eNOS and reduction of endothelial NO, leading to vasoconstriction and ischemia [70–72,84]. In this regard increasing our understanding on PON1 interaction with high density lipoproteins, its kinetics and its fate is coming to the forefront of research on such a complex particle and may yield new insights in atherosclerosis research.

7. Conclusion and perspectives

Understanding the kinetics and function of PON1 becomes an important issue in atherosclerosis. Low PON1 activity has been consistently linked with an increased risk of major cardiovascular events in the setting of secondary prevention of CAD. PON1 circulates mainly bound to HDL, and only small fractions are free in serum or bound to VLDL and chylomicrons. Only about 1/10 of HDL particles contain PON1, which suggests a micro-heterogeneity that deserves exploration. Which HDL subclasses contain PON1? How does PON1 get in HDL and what is its fate upon HDL maturation? What other lipoproteins harbor PON1? All these questions are important since our current clinical assessment of HDL relies essentially on HDL-C, a static mass assay, which has led to the current HDL Gordian knot. HDL assessment as predictor of CAD risk must evolve and progress through these steps: a) HDL-C b) HDL subclasses, c) HDL function, d) HDL function in subclasses, and e) dynamic studies, including postprandial metabolism. To gain further insight into HDL and PON1 interactions, we have developed a method that aims to assess PON1 activity in the individual HDL subclasses. We have shown that PON1 is present across the HDL particle range and preferentially in HDL_3, confirming previous data from ultracentrifugation studies. We have unraveled a large inter-individual variation in the distribution of PON1 within HDL subclasses that may have physiological significance. Upon HDL maturation ex vivo PON1 is activated as it shifts PON1 to both smaller and larger HDL particles as well as to VLDL and sdLDL. The shifts and activation are associated with shifts in apoE, AI and AII and are inhibited by CETP and LCAT inhibitors. This may partly explain the failure of CETP inhibitors to prevent cardiac events. Recent studies have shown that there is a specific interaction of MPO–apoAI–PON1 on the HDL surface that seems to be germane to atherogenesis. MPO specifically inhibits PON1 and PON1 mitigates MPO effects. These interactions if they translate to human disease will elicit a plethora of research during this decade.

Even when HDL biology is very complex, the confluence of proteomic, functional studies, HDL subclasses, PON1 assays and zymogram will yield data to draw a more elaborate and comprehensive picture of this particular function of HDL. It must be noted that all these studies are static and conducted in the fasting state. The crucial phase will be achieved when human kinetic studies (both in the fasting and post-prandial states) on HDL–PON1, apoA-I and lipid fate in the circulation are carried out. Stable isotope studies may thus ultimately clarify this gap in our knowledge. The perspectives for the future are promissory.

Conflicts of interests

None declared.

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