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The roles of PON1 and PON2 in cardiovascular disease and innate immunity

Diana M. Shih[a] and **Aldons J. Lusis**[a,b,c,*]

[a]Division of Cardiology, Department of Medicine, University of California, Los Angeles

[b]Department of Human Genetics, University of California, Los Angeles

[c]Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles

Abstract

Purpose of review—The paraoxonase (PON) gene family includes 3 members, PON1, PON2, and PON3. In vitro and mouse studies have demonstrated that all three PONs are athero-protective. Some but not all human epidemiologic studies have observed associations between PON gene polymorphisms and risk of cardiovascular disease (CVD). In this review, we summarize studies published within the past year elucidating involvement of PON1 and PON2 in oxidative stress, cardiovascular disease, and innate immune responses.

Recent findings—In a prospective study, the PON1 192QQ genotype and low PON1 activity were associated with increased systemic oxidative stress and increased risk for cardiovascular disease. PON1 expression protected against *Pseudomonas aeruginosa* lethality in *Drosophila*, suggesting that PON1 can interfere with *quorum* sensing *in vivo*. PON2 attenuated macrophage triglyceride accumulation via inhibition of diacylglycerol acyltransferase 1. Over-expression of PON2 protected against endoplasmic reticulum (ER) stress-induced apoptosis when the stress was induced by interference with protein modification but not when ER stress was induced by Ca^{++} deregulation.

Summary—Both mouse and human studies have demonstrated the anti-oxidative and athero-protective effects of PON1. The mechanisms by which PON2 exerts its athero-protective effects are emerging. Large-scale epidemiologic studies are needed to further examine the relationship between PON2 genetic polymorphisms and risk for CVD. Elucidation of the physiological substrates of the PON proteins is of particular importance to further advance this field.

Keywords

Atherosclerosis; high density lipoprotein; paraoxonases; oxidative stress; quorum sensing

Introduction

The paraoxonase gene family contains three genes, PON1, PON2, and PON3. The PON genes are located as a cluster on mouse chromosome 6 and human chromosome 7. The three human PON genes share approximately 60% similarity at the amino acid level, and about 70% similarity at the nucleotide level. Human PON1 is a 45 kDa glycoprotein expressed primarily in the liver and found associated with HDL particles in the blood [1, 2]. Human PON3 is expressed primarily in the liver, with lower expression levels in other tissues such

*Corresponding author: Aldons J. Lusis, Division of Cardiology, David Geffen School of Medicine at UCLA, 10833 Le Conte Avenue, BH-307 CHS, Los Angeles, CA 90095-1679, Phone (310) 825-1359, Fax (310) 794-7345, jlusis@mednet.ucla.edu.

as kidney [3] and the gastrointestinal tract [4]. Human PON2, on the other hand, is ubiquitously expressed and is found in a variety of tissues including the artery wall [5]. In addition, whereas PON1 and PON3 associate with HDL in circulation, PON2 protein is not associated with HDL or LDL, but appears to be associated with membranes of the endoplasmic reticulum (ER) and the nucleus [5, 6]. Although all three PON members are named “paraoxonase”, only PON1, but neither PON2 nor PON3 exhibit the ability to hydrolyze organophosphates such as paraoxon [7]. Recent studies have shown that all three PON proteins exhibit lactonase activities [7]. All three PONs very efficiently metabolized 5-hydroxy-eicosatetraenoic acid 1,5-lactone (5-HL) and 4-hydroxy-docosahexaenoic acid, which are products of both enzymatic and nonenzymatic oxidation of arachidonic acid and docosahexaenoic acid, respectively, and may represent the endogenous substrates of PONs. Interestingly human as well as mouse PONs are capable of hydrolyzing and thereby inactivating N-acyl-homoserine lactones, which are quorum-sensing signals of pathogenic bacteria such as *Pseudomonas aeruginosa* [7-9]. These studies suggest possible roles of PONs in innate immunity against bacterial infection.

Substantial epidemiological evidence points to an inverse correlation between HDL levels and coronary artery disease (CAD). One plausible hypothesis explaining this phenomenon is based on the idea that HDL can exert a direct anti-atherogenic effect at least in part by inhibiting LDL oxidation [10]. PON1 has been shown to prevent LDL oxidation *in vitro* [11-13], and decreased levels of PON1 are associated with increased risk for cardiovascular disease [14, 15]. Polymorphisms of the PON1 gene are also associated with heart disease in some but not all case-control studies [16]. In animal studies using PON1 knockout (PON1KO) mice, PON1 has been shown to be both necessary and sufficient for the *in vitro* protective effects of HDL against LDL oxidation and monocyte transmigration in response to LDL oxidation [17]. Further, PON1KO mice exhibited about a two-fold increase in atherosclerosis using both dietary and apoE-null models [17, 18], while transgenic mice overexpressing human PON1 were more resistant to atherosclerosis [19]. These studies indicate conclusively that PON1 protects against atherosclerosis.

Emerging evidence suggests the concept that PON2 is an intracellular anti-oxidative protein that decreases intracellular oxidative stress when over-expressed in various cell types [5, 6]. Polymorphism of the human PON2 gene at codon 311 (cysteine/serine) was associated with coronary artery disease and ischemic stroke [20]. Also, PON2-deficient mice showed increased diet induced-atherosclerosis as compared to the wild-type mice [21], suggesting that PON2, like PON1, protects against atherosclerosis. Below we focus on recent findings of PON1 and PON2 in the areas of oxidative stress, atherosclerosis, and innate immunity.

PON1 and cardiovascular diease

A recent prospective study of 1339 patients undergoing diagnostic coronary angiography determined PON1 activities and systemic oxidative stress at baseline and followed the participants for an average of 44 months for incidence of myocardial infarction, stroke, cardiovascular disease-related and non-related deaths. The study showed that low circulating paraoxonase activity and the PON1 QQ192 genotype are associated with increased systemic oxidative stress as measured by plasma levels of multiple, structurally specific oxidized fatty acids [22**]. The study found that compared to participants with the RR192 and QR192 genotypes, subjects with QQ192 genotype exhibited an increased risk of all-cause mortality, with an adjusted hazard ratio of 2.05, and of major adverse cardiac events, with an adjusted hazard ratio of 1.48. The incidence of major adverse cardiac events was significantly lower in participants in the highest PON1 activity quartile (7.3% and 7.7% for paraoxonase and arylesterase, respectively) compared with those in the lowest activity quartile (25.1% and 23.5%; $P<0.001$ for paraoxonase and arylesterase, respectively). The adjusted hazard ratios

for major adverse cardiac events between the highest and lowest PON1 activity quartiles were, 3.4 for paraoxonase, and 2.9 for arylesterase, and remained independent in multivariate analysis. This study demonstrated that PON1 Q192R polymorphism and PON1 activity influence systemic oxidative stress and predict prospective cardiovascular risk.

Previous reports have shown that the PON1 192Q allele is associated with endothelial dysfunction in patients with established coronary artery disease or peripheral vascular disease [23, 24]. A recent study of 99 patients with minimal atherosclerosis found that 75% of PON1 192QQ patients had endothelial dysfunction vs 43% of the PON1 192RR/QR patients ($P = 0.001$) [25*]. In PON1 192QQ vs PON1 192 Q/R and RR patients, epicardial arterial diameter decreased more and coronary blood flow increased less in response to acetylcholine. Circulating oxidized LDL levels were higher in QQ homozygotes as well. This study demonstrates that PON1 192 Q allele is associated with increased oxidative stress and endothelial dysfunction in patients with early stage atherosclerosis, providing a plausible mechanism by which this allelic variant may contribute to atherosclerosis in humans.

A recent study demonstrated association between a PON1 haplotype and risk for abdominal aortic aneurysm (AAA) [26]. This study included 423 AAA patients and 423 matched controls. The study found that a PON1 haplotype consisting of Leu at position 55, Arg at position 192 and Trp at position 194 differed in frequency between control subjects (0.374) and AAA patients (0.288) ($p < 0.042$), suggesting a protective effect of this haplotype against AAA. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been associated with AAA formation in animal models and in humans [27]. Therefore, PON1 may protect against aneurysm through its anti-oxidative function.

Aspirin therapy is an effective treatment for those with established risk factors for CVD. A recent study found that aspirin induced PON1 activity and gene expression in cultured hepatocytes and in mice [28*]. In addition, expression of apolipoprotein (apo) A-I was also increased by aspirin. The authors went on to show that this induction was mediated through the arylhydrocarbon receptor (AhR), since AhR-/- mice did not exhibit PON1 induction upon feeding of aspirin. This study suggests that part of the anti-atherosclerotic effect of aspirin may be mediated by induction of apo A-I and PON1.

PON2 and cardiovascular disease

In a recent study, PON2 mRNA and protein levels were found to be significantly lower and malondialdehyde (MDA) levels significantly higher in the atherosclerotic plaque areas of human carotid arteries, as compared to adjacent regions, fetal carotids, or mammary gland arteries [29*]. PON2 mRNA was shown to be down-regulated by oxidative stress in ex vivo experiments using segments of carotids adjacent to plaque. This study suggests that protective effect of PON2 may be reduced in atherosclerotic plaque regions due to decreased expression of PON2 in response to increased oxidative stress.

PON2 was induced by the unfolded protein response (UPR) to ER stress [6]. Furthermore, over-expression of PON2 reduced UPR-stimulated oxidative stress and apoptosis in endothelial cells [6]. A subsequent study [30*] demonstrated that PON2 overexpression protected against caspase 3 activation induced by tunicamycin or dithiothreitol, which interfere with protein modification and folding. On the other hand, PON2 failed to protect against other ER stress inducers such as thapsigargin or A23187, which disturb Ca^{++} homeostasis. Further analysis showed that ER stress caused by thapsigargin or A23187 induced Ca^{++}-dependent active degradation of PON2 mRNA and PON2 protein degradation by a Ca^{++}-dependent calpain-mediated mechanism. Therefore, the underlying cause of ER

stress determines whether PON2 expression will be induced and is likely to determine the efficacy of the cellular defense mechanisms.

Uncontrolled lipid accumulation in macrophage leads to foam cell formation. In a recent study, PON2 was shown to prevent triglyceride accumulation in macrophages [31*]. The study demonstrated elevated triglyceride (increased by 4.6 fold) but not cholesterol content in peritoneal macrophages isolated from PON2-deficient mice as compared to those isolated from the wild-type mice. Further analysis showed that the rate of triglyceride synthesis was increased in the PON2-deficient macrophages as compared to the wild-type macrophages, whereas rates of triglyceride degradation were similar between the two groups. Microsomal acyl CoA:diacylglycerol acyltransferase 1 (DGAT1) activity was significantly higher in the PON2-deficient macrophages (by 4.4 fold) as compared to the wild-type macrophages, while DGAT-1 mRNA and protein levels were similar between the two groups. Finally, the study found that incubation of PON2-deficient macrophages with a free radical generator increased cellular oxidative stress and DGAT1 activity. On the other hand, incubation of microsomes from PON2-deficient macrophages with superoxide dismutase (SOD) decreased DGAT1 activity. Therefore, PON2 seems to modulate DGAT1 activity through its anti-oxidative function.

PONs and innate immunity

All of the PON family members are capable of inactivating a bacterial quorum sensing molecule, N-3-oxododecanoyl homoserine lactone (3OC12-HSL). The specific activities of purified PON proteins toward 3OC12-HSL were in the following order: PON2≫ PON1 192R isoform > PON1 192Q isoform > PON3, with PON2 exhibiting 76 fold higher specific activity than PON3 [32*]. The study also found that the specific activity of PON2 with 3OC12-HSL was more than 2 fold higher than with 5-HL, which was previously the best substrate for PON2. By use of class-specific inhibitors, the study estimated that PON1 is responsible for about 90% of 3OC12-HSL hydrolytic activity in mouse and human serum. In mouse liver and lung homogenates, PONs appeared to be responsible for about 90% and 100%, respectively, of the 3OC12-HSL hydrolytic activities. In the human hepatoma cell line HepG2 and the endothelial cell line EA.hy 926, the 3OC12-HSL hydrolytic activities closely paralleled the PON2 protein levels after PON2 knockdown by small interfering RNA treatment of the cells. The data suggest that PONs, especially PON2, could play important role in inactivating 3OC12-HSL in mammals. In a previous study, PON2 was shown to play a dominant role in hydrolyzing 3OC12-HSL in mouse tracheal epithelial cells [33]. The study demonstrated that lysates of tracheal epithelial cells from PON2, but not PON1 or PON3, knockout mice had impaired 3OC12-HSL inactivation compared with wild-type mice. Using a quorum-sensing reporter strain of *P. aeruginosa*, the study found that quorum sensing was enhanced in PON2-deficient airway epithelial cells as compared to those of wild-type, suggesting loss of PON2 impairs 3OC12-HSL degradation by airway epithelial cells.

A previous study demonstrated inhibition of *Pseudomonas aeruginosa* biofilm formation by PON1 in an *in vitro* biofilm model [8]. A novel recent study [34**] demonstrated that transgenic expression of PON1 in *Drosophila* dramatically decreased lethality caused by *P. aeruginosa* infection. This protection was dependent on the lactonase activity of PON1 and the specific gene regulatory effects of the quorum sensing molecule in *P. aeruginosa* disrupted in the PON1 transgenic flies. This study supports the concept that PON1 plays a role in the innate immune response to quorum-sensing-dependent pathogens. Chronic inflammation caused by bacteria infection is a risk factor for CVD and it remains to be seen whether part of the anti-atherosclerotic function of PONs is dependent on their role in modulating the quorum sensing of bacteria.

Conclusion

Recent advances in the paraoxonase research field have further elucidated the protective functions of the PON family members in cardiovascular disease and innate immunity. A large-scale prospective study has provided strong evidence that PON1192QQ genotype and low PON1 activity predict future risk for CVD [22**]. Additional large-scale epidemiologic studies are needed to further determine the relationship between PON2 and PON3 polymorphisms and CVD risk. Detailed biochemical, cell-based and animal studies are needed to further identify the physiological substrates of PONs and molecular mechanisms by which PONs render their protective effects in cardiovascular disease and innate immunity.

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