Paraoxonase and Atherosclerosis

P.N. Durrington, B. Mackness, M.I. Mackness

Abstract—There is considerable evidence that the antioxidant activity of high density lipoprotein (HDL) is largely due to the paraoxonase-1 (PON1) located on it. Experiments with transgenic PON1 knockout mice indicate the potential for PON1 to protect against atherogenesis. This protective effect of HDL against low density lipoprotein (LDL) lipid peroxidation is maintained longer than is the protective effect of antioxidant vitamins and could thus be more important. There is evidence that the genetic polymorphisms of PON1 least able to protect LDL against lipid peroxidation are overrepresented in coronary heart disease, particularly in association with diabetes. However, these polymorphisms explain only part of the variation in serum PON1 activity; thus, a more critical test of the hypothesis is likely to be whether low serum PON1 activity is associated with coronary heart disease. Preliminary case-control evidence suggests that this is indeed the case and, thus, that the quest for dietary and pharmacological means of modifying serum PON1 activity may allow the oxidant model of atherosclerosis to be tested in clinical trials. (Arterioscler Thromb Vasc Biol. 2001;21:473-480.)

Key Words: paraoxonase ■ lipid peroxidation ■ high density lipoproteins

Paraoxonase-1 (PON1) is a protein of 354 amino acids with a molecular mass of 43 kDa. In serum, it is almost exclusively located on HDL. It is highly conserved in mammals but is absent in fish, birds, and invertebrates, such as arthropods. PON1 can bind reversibly to organophosphate substrates, which it hydrolyzes. In contrast, organophosphates are suicide substrates for other serum organic esterases, such as pseudocholinesterase, and for the acetylcholinesterase at synapses and the neuromuscular junction, because they bind irreversibly to them. PON1 is thus the main means of protection of the nervous system against the neurotoxicity of organophosphates entering the circulation. It was in this context that it was first discovered, and its name reflects its capacity to hydrolyze paraoxon, a metabolite of the insecticide parathion. There is wide interindividual variation in the capacity of PON1 to hydrolyze organophosphates and other organic esters.

The PON1 gene is located on the long arm of chromosome 7 between q21.3 and q22.1 with other members of its supergene family. Next to the PON1 gene is a gene that codes for 1 of the pyruvate dehydrogenase kinases and may explain the linkage of paraoxonase (PON) genotypes with diabetic glycemic control in some studies. The product of the PON1 gene is located on rabbit HDL.8

PON1 has recently emerged as the component of HDL most likely to explain its ability to metabolize lipid peroxides and to protect against their accumulation on LDL. The present review will consider first the antioxidant role of HDL in the context of its other potential antiatherogenic actions and then the evidence that PON1 is indeed responsible for the capacity of HDL to metabolize lipid peroxides before finally discussing the evidence that PON1 is linked with clinically evident atherosclerosis.

Antioxidant Role of HDL

There does not appear to be any single explanation for the inverse relationship between serum HDL and risk of atherosclerosis. For much of the time that this relationship has been known, attention has focused on the concept that HDL might be rate limiting for reverse cholesterol transport. However, evidence to support this view remains incomplete. Rabbits expressing multiple copies of the human apoA-I gene or receiving infusions of human apoA-I can be protected against experimental diet-induced atherosclerosis, but the levels of circulating apoA-I required to achieve this are greatly in excess of the variation seen in humans. When only 2 copies of the human apoA-I gene are expressed in rabbits (even so, more than doubling their HDL cholesterol concentration), they are not protected against atherosclerosis. Furthermore, apoA-I knockout mice are not rendered prone to atherosclerosis. Recent evidence concerning the cause of Tangier disease (alphalipoproteinemia) does not suggest that the profound defect in reverse cholesterol transport associated with the condition is the consequence of the low circulating
HDL but rather that its cause is mutation of the ATP binding cassette-1 transporter gene, with the low HDL representing a secondary effect of diminished cellular cholesterol efflux.14–18 Evidence is strong that low HDL cholesterol is a marker for the presence of a small, dense, cholesterol-depleted LDL in the circulation, which itself increases the risk of atherosclerosis, probably because of its susceptibility to oxidation.19 Low HDL may be linked with the generation of this type of LDL through the increased triglyceride pool that is also often present, because lipoprotein lipase activity, which is necessary to generate HDL components from triglyceride-rich lipoproteins and to catabolize them, is frequently diminished.20,21 Additionally, enhanced cholesteryl ester transfer protein activity and increased hepatic lipase activity, which are also linked with the generation of small LDL, contribute to its association with low HDL cholesterol. This is because cholesteryl ester transfer protein promotes the movement of cholesteryl ester out of HDL, and hepatic lipase can increase the hepatic uptake of HDL lipids.22–24 Again, however, the low circulating HDL cholesterol is itself simply a marker of these other metabolic processes and does not itself directly accelerate atherogenesis.

Seeking to find a more direct link between HDL and atherogenesis and with the growing evidence that the oxidation of LDL is a major factor in human atherosclerosis,25 we hypothesized that HDL might directly protect LDL against oxidative modification. At the time, it had been reported that HDL lipid peroxides transferring from a polyunsaturated fatty acyl group in the Sn2 position. In human LDL, this group is most likely to be linoleate. The most susceptible site for hydrogen abstraction and peroxidation by oxygen-derived free radicals would then be the double bond at carbon 9 in the hydrocarbon chain of the linoleate group. HDL probably catalyzes hydrolysis of the hydroperoxide at this site, releasing a carbon 9 fragment. HDL also has the capacity to remove by hydrolysis the carbon 9 fatty acid remaining at the Sn2 position of phosphatidylincholine and, thus, to leave lysolecithin.24 Of course, carbon 9 aldehydes or ketones spontaneously released from the linoleate hydroperoxide are what are believed to adduct covalently to amino acids of apoB, leading to its fragmentation and recognition by scavenger and other oxidized LDL receptors.35 However, the rapid enzymatic release of these fragments on HDL rather than LDL appears to protect apoB.36 The lysolecithin released by the action of HDL is also potentially cytotoxic. Again, however, its release on HDL is not apparently damaging. That HDL is a safe place to release lysolecithin is also strongly suggested by the huge quantities, which are known to be released there physiologically by the action of lecithin-cholesterol acyltransferase (LCAT), located on LDL. In the human, this is the main mechanism by which plasma cholesterol is esterified, including most of that newly synthesized and secreted into the circulation by the liver.37 There are numerous studies showing that HDL prevents the uptake of LDL by macrophages and other cells and reduces the cytotoxicity, which would occur under similar oxidizing conditions in the absence of HDL.26,27,30,31 In addition to glycerophospholipid peroxides, PON1 also metabolizes peroxides of cholesteryl esters.32 The PON3 protein recently isolated from rabbit serum was also shown to diminish lipid peroxide accumulation on LDL.9

The oxidant hypothesis of atherosclerosis has thus far been tested in clinical trials by attempting to increase the fat-soluble antioxidant vitamins present in lipoproteins, and results have been generally disappointing.38 However, the protection that the fat-soluble antioxidants afford LDL against lipid peroxidation is short-lived. The lag phase in conjugated diene formation, which occurs early in LDL oxidation, is the phase most clearly prolonged by fat-soluble antioxidants, but even large doses extend it only briefly, with no effect on the subsequent generation of lipid peroxides29 (Table 1). HDL, on the other hand, decreases the accumulation of lipid peroxides on LDL over several hours.29 Furthermore, the effect of the incorporation of fat-soluble antioxidants, such as vitamin E, into lipoproteins may be to increase cholesteryl ester transfer protein activity,39 which is increasingly regarded as potentially atherogenic.23 This would counteract the theoretically favorable, although limited, protection of LDL against oxidation by fat-soluble antioxidants. In any case, when these are oxidized, they themselves become
TABLE 1. Effect of Antioxidant Supplementation in Healthy Volunteers on Early Lag Phase in Conjugated Diene Formation and Later Accumulation of Lipid Peroxides on LDL When Incubated With Cu^{2+} in Presence and Absence of HDL

<table>
<thead>
<tr>
<th>Cu^{2+}-Induced LDL Oxidation</th>
<th>Conjugated Diene Lag Phase, min</th>
<th>Lipid Peroxide Accumulation Over 6 Hours, nmol/mg LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No HDL</td>
<td>HDL Present</td>
</tr>
<tr>
<td>Before vitamin supplementation</td>
<td>63.3±9.2</td>
<td>128.7±15.3</td>
</tr>
<tr>
<td>After vitamin supplementation</td>
<td>132.0±39.0*</td>
<td>136.2±14.3</td>
</tr>
</tbody>
</table>

Values are mean±SD. Lipid peroxide accumulation on HDL is unaffected by antioxidants and has been subtracted from the total lipid peroxides formed when LDL and HDL are both present.\(^{(29)}\)

\(*P<0.001\) vs value before supplementation; † \(P<0.001\) vs value with no HDL present.

pro-oxidant in the process, unless they can hand on the electrons they acquire during oxidation to another reducing agent, such as ascorbate or urate, which may not be possible in the atherosclerotic plaque.

**PON1 and Other Enzymatic Activities of HDL**

Of the proteins present on HDL that possess enzymatic (usually hydrolytic) activity (Table 2), we advanced the hypothesis that PON1 in the human was principally responsible for the breakdown of lipid peroxides before they could accumulate on LDL.\(^{(28,29,40)}\) This hypothesis was originally based on our finding that purified PON1 was highly effective in preventing lipid peroxidation of LDL,\(^{(28,40)}\) which has since been confirmed.\(^{(31,41–45)}\) In our experiments, PON1 was substantially more effective than was LCAT or apoA-I in protecting LDL against oxidation, although the combination of all 3 did slightly enhance the effect of PON1 alone\(^{(42)}\) (Figure 2). Platelet-activating factor (PAF) acetyl hydrolase (PAFAH) has an action resembling that postulated for PON1. However, PON1, like PAFAH, can also release acetate from the Sn2 position of PAF by hydrolysis.\(^{(46)}\) Although PAFAH is undoubtedly present in LDL, in our view it is not established that the PAFAH activity of HDL is due to anything other than LCAT or apoA-I in protecting LDL against oxidation, although the combination of all 3 did slightly enhance the effect of PON1 alone.\(^{(42)}\)

**Clusterin has likewise been proposed as a protein protecting cell membranes.**\(^{(50)}\) HDL is the most abundant protein in the tissue fluid and, indeed, the only lipoprotein in the central nervous system. That PON1 is present in the tissue fluid can be inferred from its presence in blister fluid.\(^{(51)}\) It is unlikely that its antioxidant function has evolved to protect humans against the oxidizing conditions alone and in the presence of apoA-I (AI), LCAT, PON1, and combinations of these.\(^{(42)}\)

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**Refer to References 34 and 46.**
cell membrane. Although PON1 was discovered as the result of its ability to hydrolyze xenobiotic toxins, there are natural organophosphate toxins\(^5\) and numerous other exogenous and endogenous esters, such as homocysteine thiolactone,\(^5\) other lactones, and cyclic carbonates,\(^5\) which it can detoxify by catalyzing their hydrolysis.\(^5\)

**Sources of Variation in PON1 Activity**

Serum PON1 activity and concentration are correlated with the HDL cholesterol and apoA-I concentration in most healthy populations studied, but the relationship is not a strong one,\(^5\) which is compatible with the PON1-containing HDL being a subspecies, the concentration of which can vary considerably independently of the major part of HDL. In extreme examples of HDL deficiency, such as Tangier disease and fish eye disease, serum PON1 is profoundly diminished,\(^5\) but in other HDL deficiencies, this is not always the case.\(^6\) This may be relevant to why some HDL deficiency states are associated with premature coronary heart disease (CHD), but others are not. What is certain, however, is that there are major influences on serum PON1 activity that are concentration independent of those governing HDL as a whole. PON1 has 2 amino acid polymorphisms, one at position 55 (methionine/leucine, M/L) and the other at position 192 (arginine/glutamine, R/Q).\(^6\) Paraoxon hydrolytic activity is greatest with HDL and with purified PON1 from PON1 192 RR and PON1 55 LL individuals and least with PON1 192 QQ and PON1 55 MM individuals.\(^6\) Heterozygotes have intermediate levels of activity. A similar pattern of substrate specificity is observed with some other oxons, such as methyl paraoxon and chlorthion-oxon, and with armine.\(^6\) On the other hand, the capacity of paraoxonase alloenzymes to protect LDL from oxidation is the complete reverse of that of paraoxon hydrolytic activity. Thus, PON1 55 MM/PON1 192 QQ individuals have HDL and PON1 associated with the greatest protective capacity.\(^4^1,4^3,4^4\) These alloenzymes are also most active in hydrolyzing diazoxon and the nerve gases sarin and soman.\(^6\) There is yet another group of substrates, such as phenyl acetate, chlorpyrifos oxon, and 2-naphthyl acetate, against which all the alloenzymes of PON1 have a similar hydrolytic activity.\(^6\)

Healthy populations in different countries also have different serum PON1 activity, which varies not simply with genotype distribution in those countries but also independently of genotype.\(^2\) Nutritional differences may well be the explanation, but thus far, there is little experimental evidence for this. In wild-type rabbits and transgenic rabbits expressing human apoA-I, changing from standard laboratory chow to a cholesterol-rich diet markedly decreases serum PON1 activity.\(^1^2\) Degraded cooking oil has been reported to lower serum PON1 in humans,\(^6^5\) and alcohol has been reported to raise it.\(^6^6\) We also have preliminary data to suggest that Gulf War veterans have low serum PON1 activity that is not explained by genotype distribution, perhaps because exposure to chemicals (possibly organophosphates themselves) may cause a long-term decrease in serum PON1 activity.\(^6^7,6^8\)

Some experimental evidence suggests that a decrease in serum PON1 activity may occur as part of an inflammatory response.\(^6^9–7^1\) It is interesting to speculate that not only might a chronic decrease in PON1 activity increase susceptibility to atherosclerosis but that more acute declines due to some intercurrent acute inflammatory condition could exacerbate LDL oxidation and, thus, foam cell generation in a critical part of a preexisting atheromatous lesion, which may weaken its fibrous cap, predisposing it to rupture and to an acute ischemic event due to clotting on the torn surface of the lesion.

### PON1 and Atherosclerosis

Numerous studies have been conducted to determine whether people with the PON1 192 R allele are more prone to CHD than those with the Q allele. These have all reported that either this is the case or that there was no association with either of the PON1 192 alleles.\(^7^2–8^8\) We have recently conducted a meta-analysis (Figure 3) that reveals a statistically significant overall association between the PON1 192 R allele and the presence of CHD if the Q allele of PON1 is more protective against CHD than is the R allele, as expected. There are also reports that the PON1 R allele increases the likelihood of CHD occurring by increasing susceptibility to other established risk factors, such as diabetes mellitus,\(^7^2\) cigarette smoking,\(^8^9\) and age.\(^9^0\) Some
other studies have also shown an association between the PON1 55 L allele and atherosclerosis,91–94 although others have not.95,96 It should be noted that all these studies have been case-control studies and that, as yet, there are no prospective investigations. However, most criticism should be reserved for claims that an association between CHD and PON1 genotype would be a valid test of the hypothesis that PON1 protects against CHD. This is because there is substantial interindividual variation in PON1 activity, which is independent of the 55 or 192 polymorphisms. Thus, there are many individuals whose serum PON1 activity is low with respect to all substrates. This may be due to acquired factors acting on the composition of the lipid environment of HDL, in which PON1 operates, or on the promoter region of the PON1 gene,97 or in some manner as yet unidentified. When PON1 activity is measured directly in patients with CHD, it is approximately half that of disease-free control subjects98–100 (also B. Mackness, unpublished data, 2001). This appears to be the case even within a few hours of the onset of cardiac ischemic chest pain in survivors of myocardial infarction, suggesting that low serum PON1 activity may have preceded the event.99 Low serum PON1 activity independent of genotype has been reported with diseases, which are known to be associated with CHD, such as diabetes mellitus,7,101–104 hypercholesterolemia,101 and renal failure.88,105–107 In the case of diabetes, the serum PON1 activity is decreased even before the onset of clinical CHD7 and in animal models of diabetes,108 we have shown that PON1 immunoreactivity is increasingly present in the arterial wall as atheroma advances.109 At present, there is no way of knowing whether this is part of a protective response, but a recent study has shown that PON1 has the ability ex vivo to hydrolyze lipid peroxides within human carotid and coronary atheromatous lesions.110

Potential for Modifying Serum PON1 Activity

As was previously discussed, nutritional effects on serum PON1 activity may prove rewarding to study. There is also considerable interest in the potential pharmacological effects on PON1 activity of the lipid-lowering drugs, fibric acid derivatives have been reported to raise serum PON1 activity in 2 studies,111,112 but this effect was not found in 2 other studies.113,114 Statin therapy may raise PON1 activity.115 In mice, polyphenols have been reported to increase serum PON1 activity,116 but this interesting group of compounds has not, so far, been studied in humans in this context.

Other Diseases Associated With Low Serum PON1 Activity

Susceptibility to organophosphate toxicity is highly likely to be related to PON1 activity and genotype.117 Most organophosphates are neurotoxins. Their acute effects in blocking neuromuscular transmission are well known, but chronic low-level exposure can produce neuropathy and perhaps neuropsychiatric effects.118 Sheep-dip workers and other industrial groups exposed to organophosphates are being studied in this regard to establish whether those with lower serum PON1 activity are more susceptible. There also remains the possibility that low serum PON1 activity predisposes one to other neurological disorders, perhaps because of the susceptibility to exposure to neurotoxins that is encountered in the course of everyday life (and certainly organophosphates are widely encountered in the diet and household) or because lipid peroxidation is a factor in the pathogenesis of disorders other than atherosclerosis. Thus, low serum PON1 activity is encountered in diabetic neuropathy (and other microvascular disease) even in the absence of clinically evident CHD,7 and the PON1 gene is linked with diabetic retinopathy.119 There are conflicting reports of an association between PON1 and Parkinson’s disease.120,121

Conclusion

PON1 would thus seem worthy of further study as an etiologic factor in the development of CHD and perhaps other diseases. Additional information is required particularly about nutritional and pharmacological effects on serum PON1 activity that might lead to intervention trials to test its capacity to prevent atheroma. Information from prospective cohort studies may also be valuable, as would a more detailed knowledge of the basic biochemistry of PON1 action and its interrelations with other HDL enzymes.

Acknowledgments

This work received support from the British Heart Foundation, Medical Research Council, and National Health Service Research and Development Levy. We are grateful to C. Price for expertly preparing this manuscript and to Drs C. Roberts and E. Hill of the Biostatistics Unit of the University of Manchester School of Epidemiology and Public Health for performing the meta-analysis.

References


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doi: 10.1161/01.ATV.21.4.473

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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