ABSTRACT

Since the discovery of human serum paraoxonase (PON1), the enzyme has been the subject of various fields of research. Initially, PON1 was identified as an enzyme capable of hydrolysing organophosphate compounds, but there is a growing body of evidence that PON1 plays a role in lipid metabolism and the onset of cardiovascular disease. Still, the precise mechanism by which PON1 functions in vivo remains to be clarified. Here we will briefly review developments in the field of PON1 research which merit further attention.

KEYWORDS

PON1, HDL, LDL oxidation, cardiovascular disease

A BRIEF HISTORY OF PARAOXONASE

In 1946, Abraham Mazur was the first to report the presence of an enzyme in animal tissue which was able to hydrolyse organophosphate compounds. This led to the initial identification of the human serum paraoxonase (PON1) enzyme in the early 1950s. PON1 was named after its ability to hydrolyse the organophosphate substrate paraoxon (paraoxonase activity, EC 3.1.8.1), which is the toxic metabolite of the insecticide parathion. Because PON1 could also hydrolyse aromatic esters, such as phenylacetate (arylesterase activity, EC 3.1.1.2), the term ‘A-esterase’ was introduced for the enzyme hydrolysing both compounds. This led to much discussion during the following years as to whether one enzyme or two were responsible for the paraoxonase and arylesterase activity, but finally, conclusive evidence was delivered that both paraoxonase activity and arylesterase activity were properties of PON1. When Mackness and colleagues demonstrated that PON1 could prevent the accumulation of lipoperoxides in low-density lipoprotein (LDL), thus linking PON1 to cardiovascular disease, the scientific interest in PON1 increased immensely. Despite the boom in research, to date the exact physiological function of PON1 is still unclear.

PON1 FAMILY

PON1 belongs to the family of serum paraoxonases, consisting of PON1, PON2 and PON3. The genes coding for these enzymes are all located next to each other on the long arm of chromosome 7 (7q21.3-q22.1). PON1 and PON3 are expressed in the liver and excreted in the blood where they are associated with the high-density lipoprotein (HDL) particle. PON2 is not present in blood, but is expressed widely in a number of tissues, including the liver, lungs, brain and heart. Of the paraoxonase family, PON1 is the most investigated and best understood member.

Recently, the crystal structure of a recombinant PON variant was solved, making PON the first HDL-associated protein of which the three-dimensional makeup has been elucidated. PON is a six-bladed β-propeller, each blade consisting of four β-sheets, and contained in the central

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Since the compounds that can be hydrolysed by PON1, e.g. organophosphates (paraoxon and diazoxon), warfare agents (soman and sarin) and aromatic esters (phenyl acetate) are nonphysiological substrates, these activities are not likely to be the physiological functions of PON1. Recent investigations have suggested that the hydrolytic activity towards lactones (cyclic esters) is the native activity of PON1: structure-activity studies show that lactones are PON1’s preferred substrate for hydrolysis. In addition, all members of the PON family have lactonase activity, implying that this activity has been conserved throughout the evolution of the enzyme. In vivo, there is a wide inter-individual variation in PON1 concentration and activity. This variation is for a major part determined by common genetic variants (polymorphisms) in the PON1 gene. Four polymorphisms in the promoter region of the PON1 gene (−107C>T, −162A>G, −824G>A, −907G>C) have been reported to affect the expression and thus the serum concentration of the enzyme. The −107C>T polymorphism has been the most important genetic determinant of PON1 levels. The coding region of the PON1 gene contains two polymorphic sites: a leucine (L) to methionine (M) transition at position 55 (55L>M), and a glutamine (Q) to arginine (R) transition at position 192 (192Q>R). Due to linkage with polymorphisms in the PON1 promoter region, the 55L>M polymorphism affects the enzyme concentration. In addition, the 55L>M polymorphism is located in the N-terminal side of PON1, which plays a role in the binding of PON1 to HDL, and may thus alter the ability of PON1 to form a complex with HDL. The 192Q>R polymorphism is responsible for a striking substrate specific change in the hydrolytic activity of the enzyme. Paraoxon is most efficiently hydrolysed by the 192R isoform and diazoxon, soman and sarin are more efficiently hydrolysed by the 192Q isoform. The capacity of blood to hydrolyse paraoxon (paraoxonase activity) is often used as a marker for the PON1 enzyme activity. This enzyme activity reflects the combined effects of the 192Q>R polymorphism and the variation in concentration of the PON1 enzyme. In addition to the paraoxonase activity, the PON1 concentration can be measured directly in serum with an enzyme-linked immunosorbent assay (ELISA). Otherwise, because PON1 esterase activity is not polymorphic (i.e. influenced by the 192Q>R polymorphism), the PON1 concentration can be estimated by measuring the arylesterase activity.

The 192Q>R and −107C>T polymorphisms are responsible for an up to 13-fold interindividual variation in PON1 enzyme activity and concentration. Lifestyle factors such as smoking and alcohol consumption also influence the PON1 in vivo status. Cigarette smoke inhibits PON1 activity in vitro, and in agreement, paraoxonase activity is lower in smokers than in nonsmokers. Furthermore, moderate consumption of beer, wine or spirits is associated with an increased serum PON1 activity.

THE ROLE OF PON1 IN HUMANS

To date, the role of PON1 in vivo is unclear, but in general, PON1 is thought to attenuate the oxidation of LDL. This hypothesis was based on in vitro findings, showing that purified PON1 inhibited the accumulation of lipid peroxides in LDL. In the arterial wall the oxidised LDL particle (oxLDL) is recognised by oxLDL specific receptors on the macrophage and taken up into the cell. Since there is no negative feedback mechanism for this uptake, this process eventually leads to an overload of lipids in the macrophage, which causes the lipid laden macrophages to aggregate and form a fatty streak characteristic of atherosclerosis. The oxidation of LDL is a key process in the pathophysiology of atherosclerosis and the onset of cardiovascular disease, and therefore, it is not surprising that PON1 has been the subject of increasing scientific interest since its alleged role in the oxidation of LDL.

Apart from inhibition of LDL oxidation, there is evidence from animal and in vitro models that paraoxonase can protect the HDL particle from oxidation and preserve the integrity of HDL. Furthermore, many epidemiological studies have found that polymorphisms in the PON1 gene, responsible for the variations in PON1 activity and concentration, also contribute to variation in plasma levels of HDL-C in different populations. Because HDL has many athero-protective functions, such as the removal of excess cholesterol from tissues (reverse cholesterol transport) and the inhibition of inflammatory processes, the preservation of the HDL particle may be a beneficial role of PON1. In blood, PON1 can hydrolyse homocysteine thiolactones, a metabotile of homocysteine. Homocysteine thiolactones can have an adverse effect on protein synthesis and may lead to endothelial dysfunction and vascular damage. The detoxification of the homocysteine thiolactone may therefore be a cardioprotective function of PON1.
Other interesting discoveries with respect to PON1 come from the field of pharmacology. The LDL-cholesterol-lowering HMG-CoA reductase inhibitors (statins) have been found to affect PON1 activity, concentration and gene expression.\textsuperscript{44-46} Reversely, since PON1 significantly predicted changes of HDL cholesterol during statin treatment in a number of populations,\textsuperscript{57,58} PON1 may be an important effect modifier of the success of the statin treatment.

**PON1 and Cardiovascular Disease**

As mentioned earlier, the finding that PON1 has properties to inhibit LDL oxidation in vitro implicated that PON1 could have a protective role in the onset of cardiovascular disease. However, the validity of those findings has been questioned since it could not be excluded that the protection against in vitro oxidation was caused by the detergent used during the preparation or a low-molecular mass compound copurified with PON1.\textsuperscript{49} Still, the results from animal experimental work uniformly show that PON1 is a protective enzyme against atherogenesis: PON1 deficiency in mice results in increased oxidative stress in serum and macrophages,\textsuperscript{59} and HDL isolated from PON1-deficient mice did not protect LDL from oxidation,\textsuperscript{60} whereas HDL isolated from human PON1 transgenic mice (having two- to four-fold increased PON1 plasma levels) was more protective against LDL oxidation in a dose-dependent manner.\textsuperscript{61} Finally, and perhaps the strongest evidence that PON1 plays a role in atherogenesis, PON1 deficient mice are more prone to develop atherosclerosis than wild-type mice, when fed a high-fat/high-cholesterol diet.\textsuperscript{19}

In humans, however, the role of PON1 genetic variants, levels and activities and the onset of cardiovascular disease is less clear. Many epidemiological studies have reported conflicting results,\textsuperscript{29} and a recent meta-analysis among 43 investigations studying the 55L>M, 192Q>R and –107C>T polymorphisms in relation to coronary heart disease (CHD), demonstrated no effect for the 55L>M and –107C>T polymorphisms and a slightly increased risk for carriers of the R-allele at position 192.\textsuperscript{54} In general, however, the effects of single genetic variants to the onset of complex diseases (such as cardiovascular disease) are often too weak to be detected in studies of relatively small sample sizes.\textsuperscript{35} It is therefore recommended to measure PON1 activity and concentration in addition to PON1 genotype.\textsuperscript{55-58} So far there have been only a few studies (the majority being case-control studies) that have measured PON1 activity and concentration.\textsuperscript{59} Furthermore, a major limitation of measuring PON1 in case-control studies is that blood is drawn after the cardiovascular event has taken place. In this way it is not possible to distinguish whether PON1 activity was the cause of the event or, conversely, a reflection of the event itself. To overcome this problem a prospective study design is needed. Up to now, only one prospective investigation on PON1 activity and concentration and CHD outcome has been published. This study showed that low serum PON1 activity toward paraoxon was an independent risk factor for coronary events in men with pre-existing CHD.\textsuperscript{59}

**Conclusion**

Despite 60 years of research, the exact role of PON1 in the human body is still unclear. An important question which still needs to be answered is whether PON1 plays a role in the onset of cardiovascular disease. Promising research themes, such as the association with the lipid metabolism and the ability to hydrolyse homocysteine thiolactones support such an involvement and merit further investigation. Until now, however, most of our knowledge on the relationship of PON1 with cardiovascular disease is based on single gene association studies, and in general, the results of genetic association studies in complex diseases have been disappointing. Therefore, it is crucial that, before drawing definitive conclusions, the contribution of the PON1 protein rather than the genetic variants should be investigated in prospective studies.

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**References**

2. Leviev I, Deakin S, James RW. Decreased stability of the M54 isoform of paraoxonase.


27. James RW, Leviev I, Righetti A. Smoking is associated with reduced serum paraoxonase activity and concentration in patients with coronary artery disease.


24. Eckerson HW, Wyte CM, La Du BN. The human serum paraoxonase/arylesterase gene (PON) status requires more than genotyping.


27. James RW, Leviev I, Righetti A. Smoking is associated with reduced serum paraoxonase activity and concentration in patients with coronary artery disease.


27. James RW, Leviev I, Righetti A. Smoking is associated with reduced serum paraoxonase activity and concentration in patients with coronary artery disease.


49. Teiber JF, Draganov DI, La Du BN. Purified human serum PON1 does not protect LDL against oxidation in the in vitro assays initiated with copper or AAPH. J Lipid Res 2004;45:2260-8.


