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REVIEW

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Pharmacogenetics of paraoxonases: a brief review

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Abstract The human paraoxonase (PON) gene family consists of three members, *PON1*, *PON2*, and *PON3*, aligned next to each other on chromosome 7. By far the most-studied member of the family is the serum paraoxonase 1 (PON1), a high-density lipoprotein-associated esterase/lactonase. Early research focused on its capability to hydrolyze toxic organophosphates, and its name derives from one of its most commonly used in vitro substrates, paraoxon. Studies in the last 2 decades have demonstrated PON1's ability to protect against atherosclerosis by hydrolyzing specific derivatives of oxidized cholesterol and/or phospholipids in oxidized low-density lipoprotein and in atherosclerotic lesions. Levels and genetic variability of PON1 influence sensitivity to specific insecticides and nerve agents, as well as the risk of cardiovascular disease. More recently, the other two members of the PON family, PON2 and PON3, have also been shown to have antioxidant properties. A major goal in present research on the paraoxonases is to identify their natural substrates and to elucidate the mechanism(s) of their catalytic activities.

Keywords Arylesterase · Lactonase · Polymorphism · Pharmacogenetics

Introduction

Mazur (1946) was the first to describe the enzymatic hydrolysis of organophosphorus compounds by animal tissues, and, during the 1950s, Aldridge (1953a, 1953b) studied the hydrolysis of paraoxon in human blood serum and sera from several other mammalian species. Aldridge proposed that these esterases, capable of hydrolyzing organophosphates as well as aromatic esters, such as *p*-nitrophenyl acetate, be called "A-esterases" (e.g., paraoxonase), to distinguish them from the "B-esterases", represented by the serine carboxylesterases and cholinesterases that are inhibited by paraoxon and other organophosphates. Human serum paraoxonase (PON1) can hydrolyze the toxic oxon metabolites of a number of insecticides such as parathion, diazinon and chlorpyriphos (La Du 1992), and even nerve agents such as sarin and soman (Broomfield and Ford 1991, Davies et al. 1996; see Fig. 1a, b). The presence of the PON1 enzyme in the blood, liver and some other tissues should provide some protection against chronic exposure to low levels of organophosphates, and have the advantage that the enzyme acts catalytically, rather than binding them stoichiometrically as the B-esterases do. However, PON1's catalytic efficiency with most organophosphates is low (see Table 1), so enzymatic hydrolysis provides only limited protection against acute exposure. For example, paraoxon given to mice lacking PON1 has about the same toxicity as it has in wild-type mice (Li et al. 2000). However, with some other organophosphates, such as diazoxon and chlorpyrifos oxon, PON1 has 10 and 20 times higher catalytic efficiencies, respectively, than with paraoxon, so PON1 seems to make a significant difference in their toxicities (Li et al. 2000).

Among the aromatic ester substrates for PON1 are phenylacetate, thiophenylacetate, and 2-naphthylacetate (La Du 1992; Fig. 1c). A variety of aromatic and aliphatic lactones as well as cyclic carbonates are also hydrolyzed by PON1, e.g. homogentisic acid lactone, dihydrocoumarin, γ-butyrolactone, and homocysteine thiolactone (Billecke et al. 2000; Jakubowski 2000) (Fig. 1d). PON1 also catalyzes the reverse reaction, lactonization, of γ- and δ-hydroxycarboxylic acids (Teiber et al. 2003).

In 1996, it was established that the gene responsible for paraoxonase/arylesterase activities described above (PON1), is a member of a multigene family (Primo-Parmo et al. 1996). Three such esterases (PON1, PON2 and PON3) have been identified to date, and these were named in the order of their discovery. Human PON2 and PON3 lack, or have very limited, paraoxonase and arylesterase activities, but are similar to PON1 in that both hydrolyze aromatic and

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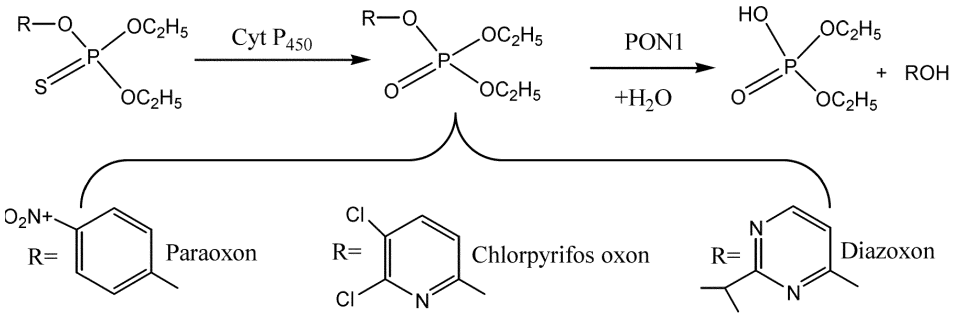
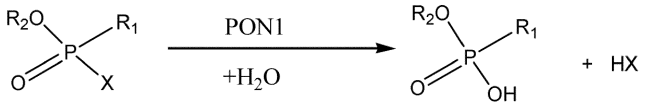
Fig. 1a–d Enzymatic activities and representative substrates for human paraoxonase-1 (hPON1)

1-a. Hydrolysis of oxon metabolites of commonly used insecticides



1-b. Hydrolysis of chemical warfare agents ("nerve gases")

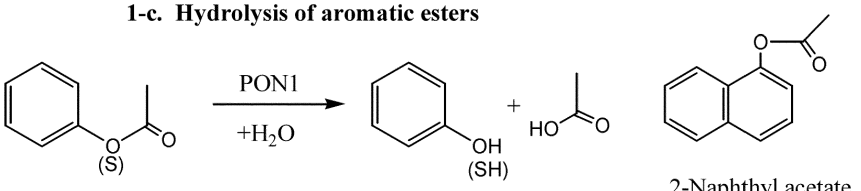


R_1= $N(CH_3)_2$ R_2= CH_2CH_3 X= CN Ethyl N-dimethylphosphoroamidocyanide (Tabun)

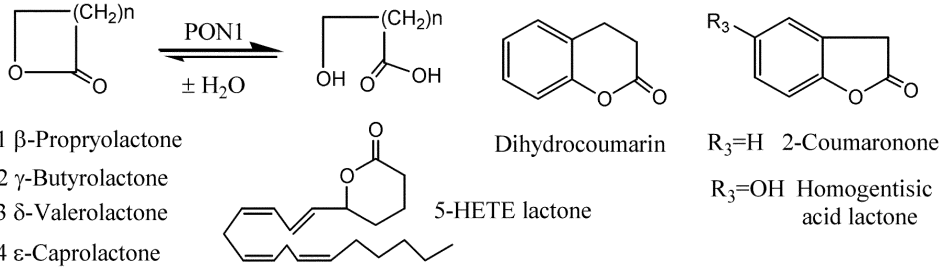
R_1= CH_3 R_2= $CH(CH_3)_2$ X= F Isopropyl methylphosphonofluoridate (Sarin)

R_1= CH_3 R_2= $CH(CH_3)C(CH_3)_3$ X= F Pinacolyl methylphosphonofluoridate (Soman)

1-c. Hydrolysis of aromatic esters



1-d. Lactone hydrolysis and lactonization of hydroxy acids



long-chain aliphatic lactones, e.g. dihydrocoumarin and 5-hydroxy-6*E*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid (5-HETE) lactone (D. Draganov, J. Teiber, B.N. La Du, unpublished data). Since lactones are common constituents of plants, and are natural flavoring agents in many food products, lactonase activity may represent an important common feature of the PON enzymes. Protection against dietary and environmental lactones could even be a selective force responsible for maintaining the balanced polymorphisms found in the mammalian PON enzymes. Furthermore, a number of drugs contain lactone or cyclic carbonate moieties that are metabolized by the PONs. For example, PON1 hydrolyses the unsaturated cyclic carbonate prodrug prulifloxacin to the active quinolone antibiotic NM394 (Tougou et al. 1998), and the rate of hydrolysis seems to depend on the polymorphism at position 192 (see below). PON1's lactonase activity has been utilized in the development of locally acting glucocorticoid drugs, which undergo rapid hydrolysis and inactivation when they reach the circulation, and thus serum PON1 prevents their unwanted systemic effects (Biggadike et al. 2000). PON3 hydrolyses widely used drugs such as the statin lactones

Table 1 Kinetic analysis of substrate hydrolysis by purified human serum paraoxonase-1_{192} (*PON1*$_{192}$) allozymes

Substrate	PON1_{192Q}			PON1_{192R}			Activity ratio V_{max} Q/R
	V_{max} (U/mg)	K_m (mM)	V_{max}/K_m	V_{max} (U/mg)	K_m (mM)	V_{max}/K_m	
Phenylacetate[1]	845	0.69	1225	720	0.27	2700	1.2
Paraoxon[1]	0.47 (0.96)[2]	0.50	0.94 (1.9)[2]	2.1 (6.1)[2]	0.27	7.8 (23)[2]	0.22 (0.16)[2]
Sarin[3]	69	0.21	330	21	0.31	68	3.3
Soman[3]	82	0.42	195	31	0.25	124	2.6
γ-Butyrolactone[4]	420	15	23	290	5.3	55	1.4
Dihydrocoumarin[4]	180	0.022	8200	160	0.013	12300	1.1

[1]From Smolen et al. (1991)
[2]V_{max} in presence of 1 M NaCl in *brackets*, K_m values remained the same
[3]From Broomfield and Ford (1991)
[4]From Billecke et al. (2000)

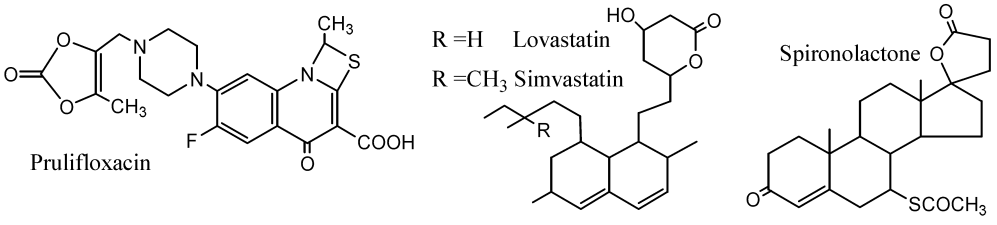


Fig. 2 Drug substrates for hPON1 (prulifloxacin) and hPON3 (lovastatin, simvastatin, spironolactone)

(lovastatin and simvastatin) and the diuretic spironolactone (Fig. 2).

Evolution of the PON Family

Augustinsson (1968) proposed that the arylesterases arose as an offshoot from the carboxylesterases, but differed by having cysteine, rather than serine, as the key component of their active centers. Although this hypothesis remained unchallenged for many years, the extensive cloning and sequencing of the hydrolytic enzymes made it evident that there is no structural similarity in the amino acid sequences of the PONs with sequences found for the mammalian cholinesterases, carboxylesterases, or bacterial organophosphatases. It was concluded that they are not related, so other ancestral relatives had to be sought for the PON family (LaDu et al. 1999a). Kobayashi et al. (1999) have cloned a lactone hydrolase from the fungus *Fusarium oxysporum*, which has appreciable structural homology with human serum PON1. Furthermore, these two enzymes share a number of substrates, such as dihydrocoumarin and homogentisic acid lactone. Interestingly, the fungal lactonase does not hydrolyze paraoxon. Other PON-like sequences can be found in bacteria, plants, the worm *Caenorhabditis elegans*, and the fruit-fly *Drosophila melanogaster*. We have cloned PON-like cDNAs from the zebrafish (*Danio regio*), frog (*Xenopus laevis*), chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*) and a number of mammals (Primo-Parmo et al. 1996; Draganov et al. 2000; D. Draganov, unpublished data). A phylogenetic tree of the vertebrate PONs is presented in Fig. 3. On the basis of the structural homology and predicted evolutionary distance between them, it appears that PON2 is the oldest member of the family; PON3 arose from it next, and, more recently, PON1 appeared.

Enzymatic characteristics and tissue distribution

Human PON1 is synthesized in the liver and secreted into the blood, where it is associated exclusively with high-density lipoprotein (HDL) (Mackness 1989a, 1989b; Hassett et al. 1991). Serum PON1 is a glycosylated protein of 354 amino acids with an apparent mass on SDS-PAGE of 43–47 kDa. The mature protein retains its hydrophobic leader sequence, from which only the initial methionine is cleaved (Hassett et al. 1991). The retained *N*-terminal signal peptide is a structural requirement for PON1's association with HDL (Sorenson et al. 1999). The enzyme has two calcium-binding sites with K_d values of 0.36 and 6.6 μM^{-1}; the higher-affinity site is essential for enzyme stability; the other is essential for enzymatic hydrolytic activity (Kuo and LaDu 1998). The exact amino acid residues participating in Ca^{2+}-binding sites remain to be defined. Selective chemical modification of aspartic acid (D) and glutamic acid (E) residues with carbodiimides prevents Ca^{2+} binding and inactivates human PON1 (Josse et al. 1999). Eighteen of the conserved acidic amino acids in human PON1 have been mutated to alanine to make single mutants, eight of which have less than 10% of the wild type arylesterase and paraoxonase activities: E53,[1] D54, D 169, D183, E195, D269 and D279 (Josse et al. 1999). Except D269, none of these lie within the three putative EF-hand-type calcium-binding sites identified in human

[1] The numbering of the amino acid residues in this review is in accord with the recommendations of the Third International Meeting on Esterases Reacting with Organophosphorus Compounds (Dubrovnik, Croatia, April 1998) (La Du et al. 1999b) and starts with the initial Met. Thus the numbering in the text may differ by +1 with some of the original references in which the numbering starts from Ala_2 (the first amino acid in the secreted protein). Also we refer to one of the PON1 promoter polymorphisms as T(–108)C rather than –107 number used by R.W. James' group.

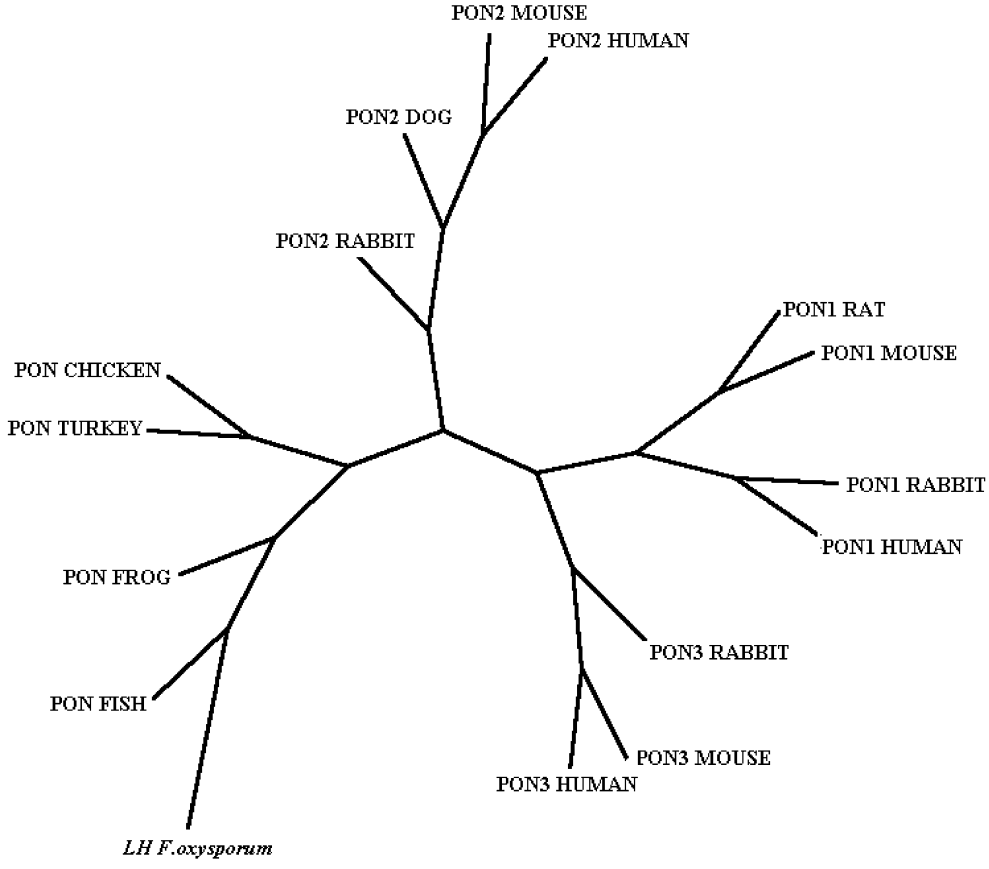


Fig. 3 Phylogenetic tree of vertebrate paraoxonases. The fungal lactone hydrolase (*LH*) was used to root the tree

and rabbit PON1 (Kuo and LaDu 1998), suggesting that Ca-binding residues are dispersed in the primary structure (Josse et al. 1999). Removal of Ca^{2+} by chelating agents irreversibly destroys PON1 activity and stability, whereas some divalent ions (Zn, Mn, Mg) can keep PON1 in a stable, although inactive form (Kuo and LaDu 1998). PON1 has three cysteine(C) residues (at positions 42, 284 and 353); the first and third form a disulfide linkage but residue 284 is free (Kuo and La Du 1995; see Fig. 4). Site-directed mutagenesis of C42 or C353 to alanine leads to PON1 inactivation and significantly reduces secretion (Josse et al. 1999). Mutating C284 to alanine or serine decreases, but does not abolish the paraoxonase and arylesterase activities (Sorenson et al. 1995b). In contrast, Aviram et al. (1998) have demonstrated that Ca^{2+} is not essential, but the C284 is required for PON1's ability to protect low-density protein (LDL) against copper-induced oxidation. This led to the speculation that PON1 possesses two catalytic sites – one for the hydrolytic activities, and another for the antioxidant activity (Aviram et al. 1998, Aviram 1999). Using group-selective labeling and site-directed mutagenesis, Josse et al. (1999) have identified additional amino acid residues essential for PON1 esterase activities: H115, H134, H155, H243 and W281.

PON2 and PON3 have been much less studied. Similar to PON1, PON3 is expressed mostly in the liver and at low levels in the kidney (Reddy et al. 2001). PON3 mRNA and protein have been identified in murine, but not in human macrophages (Rosenblat et al. 2003). Rabbit and rat PON3 have been purified from liver microsomes (Ozols 1999; Rodrigo et al. 2003). Rabbit and human PON3 are found in serum associated with HDL, but about two orders of magnitude less abundant than PON1 (Draganov et al. 2000; Reddy et al. 2001). PON3 has very limited arylesterase and paraoxonase activities, but shares many lactone substrates with PON1 (Draganov et al. 2000). Lactone hydrolysis of lovastatin, simvastatin and spironolactone reported for purified human serum PON1 (Billecke et al. 2000) was due to hPON3, present in small amounts in the PON1 preparations (D. Draganov, J. Teiber, B.N. La Du, unpublished observations). PON2 is not detectable in plasma but is expressed widely in a number of tissues, including brain, liver, kidney, testis, and may have multiple mRNA forms (Mochizuki et al. 1998; Ng et al. 2001). Dihydrocoumarin is the only substrate reported so far for PON2 (Rosenblat et al. 2003). Like PON1, both PON2 and PON3 have been shown to prevent cell-mediated oxidative modification of LDL (Ng et al. 2001; Reddy et al. 2001), but the exact substrates and mechanism of their protective activities remain to be elucidated.

PON genes

The human liver PON1 cDNA sequence has been determined by Hassett et al. (1991) and Adkins et al. (1993). The rabbit liver PON1 sequence was reported in 1991 (Hassett et al. 1991), and the mouse liver PON1 sequence

Fig. 4 Alignment of human PON1, -2, and -3 amino acid sequences. *Dashes* indicate identical amino acids as in PON1. The polymorphic sites for PON1 at positions *55*, *102*, *160*, and *192* are in *parentheses above* the sequence; an alternative spice variant for PON2 and the polymorphic sites for PON2 at positions *148* and *312* are in *parentheses below* the sequence. The cysteines forming a disulfide bridge in PON1 are indicated with *arrows*; # indicates the potential *N*-glycosylation sites. The *asterisks* indicate position *106* (present only in mammalian PON1, absent in PON2 and -3). This numbering is in accord with La Du et al. (1999b)

in 1995 (Sorenson et al. 1995a). The three PON genes are very closely aligned on chromosome 7 in man, and on chromosome 6 in the mouse (Primo-Parmo et al. 1996). In both species the three PONs contain nine exons of approximately the same length. Based on their respective cDNA structures and the deduced amino acid sequences, there is over 80% identity in amino acid residues in human, mouse and rabbit PON1 proteins, and at least 60% identity between the PON 1, 2, and 3 within each of these species (Primo-Parmo et al. 1996). All PON1s have an extra codon at position 106 (lysine in human PON1), which is missing in PON2 and PON3 cDNAs (Primo-Parmo et al. 1996). Polymorphic variants are common in at least the human and rabbit PONs (Watson et al. 2001). A reasonable conclusion from these observations is that this family of enzymes arose by gene duplication and the PONs probably have some important physiological roles that are insured by the redundancy and polymorphic forms of the proteins. The deduced amino acid sequences of human PON1, -2 and -3 are shown in Fig. 4.

Polymorphic forms of *PON1* and *PON2*

Two common polymorphisms in the coding region of hPON1 have been studied extensively in the past decade: leucine(L)/methionine(M) at position 55 and glutamine(Q)/arginine(R) at position 192. More attention has been paid to the 192 polymorphism because the two allozymes differ considerably in their affinity for and catalytic activity with a number of substrates. Paraoxon is hydrolyzed 6 times faster by the $PON1_{192R}$ allozyme than by the $PON1_{192Q}$ al-

lozyme, but some organophosphates and lactones are hydrolyzed faster by the latter (Davies et al. 1996; Billecke et al. 2000) (see Table 1).

The $PON1_{55M}$ allozyme is associated with lower serum enzymatic activity than the $PON1_{55L}$ allozyme, but it is not clear whether this is because of a decreased stability of the $PON1_{55M}$ alloenzyme, as suggested by Leviev et al. (2001), and/or because of the linkage disequilibrium with the –108T allele described below (Leviev and James 2000, Brophy et al. 2001b). At least five polymorphisms have been identified in the 5′-regulatory region of hPON1: –108(107)T/C, –126G/C, –162A/G, –832(824)G/A and –909(907)C/G with somewhat different effects on PON1 expression (Leviev and James 2000; Suehiro et al. 2000; Brophy et al. 2001a); numbering differences are indicated in parentheses, and these data are too recent for a numbering consensus to have been reached). Of these only the –162, a potential nuclear factor-1 (NF-1) binding site, and the –108, stimulating protein-1 (Sp1) binding site, lie within consensus sequences for known transcription factors (Brophy et al. 2001a). T at position –108 disrupts the GGCGGG recognition sequence for Sp1 and results in decreased affinity for hepatocyte nuclear extracts and Sp1 (Deakin et al. 2003). The polymorphisms at –108C, –162A, –832A, and –909G each affect the relative luciferase expression in vitro approximately twofold (Leviev and James 2000; Suehiro et al. 2000; Brophy et al. 2001a). These data correlate well with higher serum PON1 levels associated with these genotypes in different populations (Leviev and James 2000; Suehiro et al. 2000; Brophy et al. 2001b). The analysis of the individual contribution of each polymorphism in the promoter region on serum paraoxonase activity/levels is complicated because of the pronounced linkage disequilibrium between them and the 55M/L and 192Q/R polymorphisms in the coding region. Ultimately the –108CC genotype is associated with the highest serum PON1 levels, –108TT with the lowest, and the heterozygotes with intermediate levels (Leviev and James 2000; Suehiro et al. 2000; Brophy et al. 2001b). In addition, four "polymorphic" sites have been identified in the 3′-untranslated regions of PON1 and these remain to be characterized (Brophy et al. 2001b). More recently, two other polymorphisms in the PON1 coding region have been reported: isoleucine to valine at position 102 in Finns (Marchesani et al. 2003) and arginine to glycine at position 160 in the Chinese Han population (Wang et al. 2003). Their presence in other populations remains unknown. At present it is also not known whereas these polymorphisms have any effect on PON1's hydrolytic and/or protective activities. All together, more than 200 single nucleotide polymorphisms (SNPs) have been identified in the human PON1 gene (see: http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=5444), many of which are in strong linkage disequilibrium and form certain haplotypes within the gene (Jarvik et al. 2003). This limits the usefulness of association studies with particular PON1 genotypes as emphasized in the "PON1 status" section below.

Human PON2 also has two common polymorphic sites in the coding region: alanine/glycine at position 148 and cysteine/serine at position 311. A higher association of the $PON2_{311S}$ allele with coronary heart disease has been reported (Sanghera et al. 1998; Hegele 1999; Leus et al. 2001). However, very little is yet known about the catalytic functions of human PON2, so these clinical observations are difficult to interpret at this time.

Ethnic distribution of PON1 polymorphism

The frequency of the 192Q/R polymorphic forms of PON1 varies considerably around the world. Bimodal distribution of the levels of serum paraoxonase activity in genetic studies of several British families (Playfer et al. 1976) have indicated that the lower of the two modes represented exclusively people who were homozygous for a low-activity allele. This allowed a calculation of the frequency of both alleles (low activity and high activity) that was adequate for estimating PON1gene frequencies in the bimodal population samples of Northern Europe, Canada and the USA (La Du 1992). The low-activity phenotype has been shown subsequently to represent homozygosity for the $PON1_{192Q}$ allozyme, whereas the high paraoxonase activity phenotype represents the combination of the heterozygotes and the homozygotes for the $PON1_{192R}$ allele (Adkins et al. 1993; Humbert et al. 1993). The above paraoxonase activity phenotyping method did not distinguish adequately between heterozygotes and homozygotes for the high-activity allele (Eckerson et al. 1983), and it was not appropriate for most African, Near-Eastern and Far-Eastern population samples, since paraoxonase activities in those regions are not distributed bimodally (Geldmacher-von Mallinckrodt and Diepgen 1988; La Du 1992). Another method of phenotyping has been developed on the basis of the ratio of the paraoxonase activity (at pH 10.5 in presence of 1 M NaCl) and the arylesterase activity with phenylacetate (Eckerson et al. 1983), given that arylesterase activity is directly proportional to PON1 serum concentration, regardless of the genotype. This method gave trimodal distributions with values (ratios) for the first mode of about 1.2, for the second of around 4.7, and for the third about 8.4, corresponding respectively to the low-activity homozygotes, heterozygotes and high-activity allele homozygotes. After elucidating the molecular basis ($PON1_{Q192R}$) for paraoxonase polymorphism, this method was validated later by genotyping of the same samples (Adkins et al. 1993). Other activity ratios have also been developed and used in much the same way, for example, chlorpyrifos oxon with paraoxon (Davies et al. 1996) or diazoxon and paraoxon (Richter and Furlong 1999). Some examples follow (Table 2) to illustrate the divergent frequencies of the $PON1_{192R}$ and $PON1_{192Q}$ alleles in different ethnic and geographic regions of the world. The R allele is most frequent in Central Africa and in some aboriginal or isolated regions, whereas the Q allele has become more frequent in the temperate regions of Europe and North America. To what degree this distribution represents the migration of the populations, or the influence of the local climate and environmental factors must still be determined.

Table 2 Geographic and ethnic distribution of human PON1 polymorphisms

Geographic region		% of population with low activity[1]	Q192 allele frequency[2]	L55 allele frequency[2]	–108C allele frequency[3]
North America	Caucasian	51–52	0.70–0.72	0.64	0.50
	Afro-American	15	–	–	–
Europe (Caucasian)		53	0.67–0.74	0.57–0.64	0.46
Africa		0–20	–	–	–
Near East		35–50	–	–	–
Far East		10–20			
	Chinese	–	0.36–0.43	0.96	–
	Japanese	10	0.38–0.41	0.91–0.94	0.48
Australia	Caucasian	–	0.63	0.60	–
	Aborigines	0	–	–	–

[1]Modified from La Du (1992)
[2]Genotyping studies from multiple sources, for an extensive list of relevant references see Brophy et al. (2002)
[3]From Leviev and James (2000); Suehiro et al. (2000); Brophy et al. (2001b)

"PON1 status"

PON1 serum levels differ significantly between individuals (up to 13-fold) and are modulated by disease state, dietary, life-style, and environmental factors (Costa et al. 2003). With the technological advancements available today it is very easy to determine particular PON1 genotypes directly by DNA sequencing, PCR amplification/restriction enzyme analysis, real time fluorescent PCR with melting point analysis etc. However, the phenotyping ratio methods, outlined above, provide a direct quantitative measure of the functional effects of the usual or variant forms of the PON1 with different substrates. This additional information referred as the "PON1 status" (Richter and Furlong 1999) supplies a broader basis than the genotype, alone, for correlation with disease susceptibility, or responsiveness to environmental agents (Furlong 2000; La Du et al. 2001; Brophy et al. 2002). Thus the catalytic efficiency of each $PON1_{192}$ allozyme must be considered, as well as the *level* of that particular allozyme. $PON1_{192QR}$ heterozygous individuals have a mixture of the two isoforms, and in some heterozygotes, there will be a higher level of one of the two allelic types than is present in some individuals homozygous for that allozyme (it is incorrect to assume that all heterozygotes have one-half of the average level of each allozyme). If the two allelic forms of the enzyme differ greatly in their catalytic efficiencies, the contribution of each allelic form should be evaluated with respect to toxicity from environmental chemicals (La Du et al. 2001).

PON1 and cardiovascular disease

The special localization of the PON1 in the HDL complex of human serum led to the suggestion by Mackness (1989a, 1989b) that the enzyme might play an important physiological role in lipid metabolism and protect against the development of atherosclerosis. This speculation has stimulated many important investigations over the past decade with considerable evidence that PON1 may protect vascular tissues from oxidative damage (Aviram 1999; Durrington et al. 2001). An additional important clue to a physiological function for PON1 has been provided by studies with mice lacking this enzyme (Shih et al. 1998). The "knock-out" mice develop atherosclerosis when fed an atherogenic diet, and their HDL, in contrast to wild-type HDL, fails to prevent LDL oxidation in cultured artery wall cells (Shih et al. 1998). Purified PON1 shows the expected protection against oxidative damage from LDL (Aviram et al. 1998; Navab et al. 2002). Furthermore, purified rabbit serum PON3 has recently been found to be even more protective than PON1 against copper-induced LDL oxidation (Draganov et al. 2000). Although the lactonase activity of some model compounds seems to parallel roughly the degree of protection against oxidative damage, it is not clear whether these two activities are related directly. Perhaps protection involves the hydrolysis, and thereby the inactivation of some potentially toxic endogenous lactones. Such questions will be answered by future research. A brief summary of possible mechanisms by which PONs may protect against cardiovascular disease and atherosclerosis is given in Table 3.

Many clinical papers have been published in the past decade, most supporting, but some excluding, a relationship between the PON1 polymorphisms and the development of cardiovascular disease and atherosclerosis (reviewed in Heinecke and Lusis 1998; Hegele 1999; Durrington et al. 2001; Brophy et al. 2002; Shih et al. 2002; Costa et al. 2003). The frequent association of $PON1_{192R}$ with vascular disease predicts decreased efficiency for this allozyme in the metabolism of oxidized lipids and/or less stability compared with $PON1_{192Q}$. Evidence for both has been provided experimentally (Mackness et al. 1998; Aviram et al. 1998, 2000). However, as pointed out in the previous section, serum PON1 level/activity is also an important variable and most probably a better predictor of

Table 3 Evidence for human PON1 antiatherogenicity (summarized from Aviram 1999; Durrington et al. 2001; Navab et al. 2002)

1. Protects HDL against oxidation and preserves its functions
↑ cellular cholesterol efflux from macrophages
2. Protects LDL against oxidation
↓ lipid peroxides
3. Ameliorates effects of oxidized LDL
↓ inflammatory and cytotoxic oxidized phospholipids
↓ LDL uptake by macrophages
↓ monocyte transmigration induced by oxidized LDL
4. Decreases lipid peroxides in atherosclerotic lesions

the risk of cardiovascular disease (Jarvik et al. 2000; Mackness et al. 2001). In a recent study Jarvik et al. (2003) have demonstrated that PON activity predicts carotid artery disease, yet four PON1 functional polymorphisms do not. Future studies of low-activity PON1 patients may reveal how PON1 protects against cardiovascular disease (La Du 2003).

PON1 and other diseases

Most of the recent research efforts to determine a physiological role for PON1 (and possibly the other PONs) have focused on atherosclerosis and cardiovascular diseases. However, it is logical to extend the proposed protective mechanism of PON1 against oxidative vascular damage to other organs and tissues.

Low serum PON1 activity has been found in diabetes mellitus (both type I, insulin-dependent, and type II, non-insulin-dependent) and renal disease (reviewed in Mackness et al. 2002a). In most studies the lowest PON1 levels have been found in patients with vascular complications, but causal relationships remain to be estimated. In type-I diabetic patients low PON1 levels and activity, independent of PON1 55 and 192 polymorphisms, have been found (Boemi et al. 2001; Mackness et al. 2002b). Diabetic complications in Japanese population, such as retinopathy (Kao et al. 1998) or central retinal vein occlusion (Murata et al. 1998), may be associated with the $PON1_{55L}$ and $PON1_{192R}$ allozymes, respectively. A higher frequency of the $PON1_{192R}$ allele has been reported in type-II diabetic patients with macrovascular disease (Hu et al. 2003), cerebrovascular disease (Koch et al. 2001), and coronary artery disease (Osei-Hyiaman et al. 2001). Noteworthy is that the association of the $PON1_{192R}$ genotype with an increased risk of vascular disease is more prominent in patients with accompanying disease (e.g., diabetes) and/or in Eastern populations, in which the $PON1_{192R}$ allele is prevalent.

No association between Parkinsonism and PON1 192 polymorphism was found by a Russian laboratory (Akhmedova et al. 1999), but a significant increase in the $PON1_{192R}$ allele was noted in a Japanese study (Kondo and Yamamoto 1998). The implication in the latter study is that $PON1_{192R}$ allozyme is less effective against some environmental neurotoxins than $PON1_{192Q}$. No association of PON1 55 and 192 polymorphisms have been found for Alzheimer's disease or vascular dementia (Dantoine et al. 2002; Paragh et al. 2002; Pola et al. 2003), but PON levels are lower in patients with dementia than in age-matched controls (Paragh et al. 2003). A lower $PON1_{192R}$ frequency in patients with sporadic Alzheimer's disease, but a higher $PON1_{192R}$ frequency in patients with coronary artery disease, has been found by Scacchi et al. (2003).

Haley et al. (1999) have reported a comparison of a small number of Gulf War Veterans with neurological symptoms with unaffected or non-deployed controls showed that the level of the $PON1_{192Q}$ allozyme activity was lowest (or was $PON1_{192R}$) in those most severely affected. Different sensitivities to some neurotoxins might be explained if certain toxins are hydrolyzed more efficiently by human $PON1_{192Q}$ than by the $PON1_{192R}$ allozyme.

Systemic infection and inflammation reduce serum PON1 levels and expression (Feingold et al. 1998; Van Lenten et al. 2001; Kumon et al. 2002). These changes are accompanied with alterations in other HDL associated proteins (e.g., apolipoprotein A-I, lecithin:cholesterol acyltransferase, cholesterol ester transfer protein), and could decrease the ability of HDL to protect against atherosclerosis. Bog-Hansen et al. (1978) have suggested that PON1 might protect against bacterial endotoxins. Purified $PON1_{192Q}$ applied before or shortly after injection of mice with a lethal dose of lipopolysaccharide rescued 60% and 30% of the animals, respectively (La Du et al. 1999a). The role of PON1 in human and experimental animal models of sepsis are currently under investigation in the author's laboratory.

Conclusions

Serum paraoxonase (PON1) and the other members of the PON family of enzymes are conserved, closely related proteins of uncertain function and physiological significance. PON1's organophosphatase and arylesterase activities, which utilize mostly human-made chemicals, seem to be ancillary rather than primary function of the enzyme: natural organophosphates such as anatoxin-a, for example, are not hydrolyzed by PON1 (LaDu et al. 1999a). In contrast, lactonase/lactonizing activities utilize natural substrates and are shared by all three PONs and their homologues. This redundancy within these closely related enzymes suggests that they may share other important hydrolytic activities. Generation of PON2 and PON3 "knock-out" mice is currently underway and may help to uncover their (patho)physiological roles. The belief that PON1 has the ability to prevent oxidative damage to tissues in general seems to be a reasonable hypothesis, but little concrete evidence, except for atherosclerosis studies, is yet available to support it. A monograph on PON1's role in health and disease has been published recently (Costa and Furlong 2002).

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