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Paraoxonase 1 Status as a Risk Factor for Disease or Exposure

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Abstract

Human paraoxonase 1 (PON1) has broad substrate specificity and has been shown to protect against exposure to some organophosphorus (OP) insecticides due to its ability to hydrolyze toxic metabolites of some organophosphorothioate insecticides. PON1 status has been shown to be important in protecting against vascular disease, presumably due to the not-as-yet fully characterized role of the three PON proteins in modulating oxidative stress. More recently, all three PONs (1, 2, and 3) have been shown to inactivate the quorum sensing factor *N*-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL) of *Pseudomonas*. Expression of human PON1 in *Drosophila* demonstrated the importance of PON1 in resistance to *Pseudomonas* infection. Many studies have examined only DNA single nucleotide polymorphisms as possible risk factors for disease or exposures. For all of the known functions of PON1, the level of PON1 enzyme is important and, in some cases, also the Q192R polymorphism. A simple high throughput two-substrate assay/analysis, plotting rates of diazoxon hydrolysis vs. paraoxon hydrolysis, provided both PON1 levels and functional Q192R phenotype/genotype. We have developed a new two-substrate assay/analysis protocol that provides PON1 status without use of toxic OP substrates. Factors were determined for inter-converting rates of hydrolysis of different substrates.

Keywords

PON1 status; Paraoxonase; Diazoxon; Diazinon; Chlorpyrifos; Chlorpyrifos oxon; Carotid artery disease; Quorum sensing factor; OP exposure

1 Introduction

Human paraoxonase 1 (PON1) has broad substrate specificity and has been shown to protect against exposure to some organophosphorus (OP) insecticides due to its ability to hydrolyze their toxic oxon metabolites at physiologically relevant rates (Costa et al. 1990; Li et al. 1993, 1995, 2000; Shih et al. 1998; Cole et al. 2005). The hydrolysis of other OPs, such as paraoxon (PO), while detectable with in vitro assays, is not physiologically relevant due to insufficient catalytic efficiency of hydrolysis (Li et al. 2000). The PON1 Q192R polymorphism affects the catalytic efficiency of hydrolysis of some PON1 substrates (Davies et al. 1996; Li et al. 2000). We introduced the term PON1 status to include both the functional $PON1_{192}$ genotype as well as the plasma level of PON1, both of which can be important in determining risk of disease or exposure (Li et al. 1993). In all cases, rates of detoxication of both endogenous and xenobiotic substrates are determined by the plasma level of PON1, provided that the catalytic efficiency of hydrolysis is physiologically significant. For some cases, such as the detoxication of chlorpyrifos oxon (CPO), plasma PON1 level and the Q192R polymorphism are both important, with the $PON1_{R192}$ alloform detoxifying CPO more efficiently than

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$PON1_{Q192}$ (Li et al. 2000). The efficiency of detoxication of diazoxon (DZO) is nearly equivalent for both $PON1_{192}$ alloforms (Li et al. 2000).

PON1 status has been shown to be important in protecting against vascular disease (Jarvik et al. 2000), presumably through the role of PON1 in modulating oxidative stress (reviewed in James 2006). More recently, all three PONs (1, 2, and 3) have been shown to inactivate the quorum sensing factor *N*-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL) of *Pseudomonas* (Ozer et al. 2005). The definitive study by Stoltz et al. (2008; Chapter 17 in this book), where the expression of human PON1 in transgenic *Drosophila* resulted in increased resistance to infection by *Pseudomonas aeruginosa*, indicates that the PON family of proteins can also be considered as part of the innate immunity system (Chun et al. 2004).

After the genetic variability of PON1 was linked to cardiovascular disease, many studies have been carried out that examined only DNA single nucleotide polymorphisms (SNPs) as possible risk factors for disease or exposures. Some studies examined only the Q192R polymorphism, others both the Q192R and L55M polymorphisms and yet others have included the analysis of one or more promoter region polymorphisms, the most important of which appears to be the C-108T polymorphism that occurs in an Sp1 binding site (Deakin et al. 2003). Relatively few studies have examined the relationship between PON1 levels or activity and risk for disease. Mackness et al. (2001) found decreased levels of plasma PON1 (by ELISA) and paraoxonase (POase) activity among patients with coronary heart disease (CHD). They also carried out a meta-analysis of 18 previous studies, only three of which determined PON1 levels or activity. The assay of POase is not a good measure of risk for disease, since this activity is dramatically affected by the Q192R polymorphism with $PON1_{R192}$ having much higher POase activity than $PON1_{Q192}$. Since the gene frequencies for $PON1_{Q192}$ and $PON1_{R192}$ vary significantly among different ethnic groups, a mixture of individuals of different ethnic origin can significantly skew the data (Brophy et al. 2002). Mackness et al. (2001) recommended that “We, along with other authors, would strongly suggest that all further epidemiological studies into the role of PON1 and disease should include a measurement of the enzyme itself in addition to the genetic polymorphisms.” This same recommendation was echoed by two other experienced PON1 research teams (Deakin and James 2004; La Du 2003).

A second large study (Lawlor et al. 2004) examined association of the Q192R polymorphism with CHD in a large cohort (n = 3,266) combined with a meta-analysis of 38 other studies. These SNP analyses revealed no association with CHD; however, they suffered from the critical lack of data on plasma PON1 levels.

A third meta-analysis which examined four PON1 polymorphisms and one PON2 polymorphism included 43 genetic association studies (>11,000 cases and ~ 13,000 controls) and showed no significant association with CHD (Wheeler et al. 2000). This study also suffered from a lack of data on plasma PON1 levels or activity.

For all of the known functions of PON1, the level of PON1 is important and, in some cases, also the Q192R polymorphism. Figure 1 shows the PON1 polymorphisms and frequencies identified by the Seattle SNPs resequencing effort (Furlong et al. 2008). Characterizing all of the nearly 200 PON1 polymorphisms will not provide an accurate prediction of plasma PON1 levels. Measurement of the of the phenyl acetate hydrolysis activity of PON1 (AREase) is unaffected by the Q192R polymorphism and can serve as a surrogate measure of plasma PON1 protein levels (Furlong et al. 2006;Richter et al. 2008). Measurement of the AREase activity of plasma PON1 or determination of plasma PON1 protein levels by ELISA are the minimum measures that should be carried out in any epidemiological study. More useful measures are described below.

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2 Two-Substrate Analyses of PON1 Status

Early studies by Eckerson et al. (1983) showed that a two-substrate assay/analysis plotting rates of PO hydrolysis vs. AREase would provide both plasma PON1 levels and a separation of low ($PON1_{Q192}$ homozygotes) from the high metabolizers. This analysis, however, did not resolve $PON1_{192}$ heterozygotes from $PON1_{R192}$ homozygotes. When we made use of the two-substrate assay/analysis to examine rates of a number of different PON1 substrates, we found that plotting rates of diazoxon hydrolysis vs. paraoxon hydrolysis provided both relative PON1 levels for each $PON1_{192}$ functional genotype/phenotype as well as a clear resolution of all three $PON1_{192}$ phenotypes (Q/Q, Q/R, and R/R) (Davies et al. 1996; Richter and Furlong 1999). This analysis, however, uses two highly toxic OPs, DZO and PO.

3 Development of a PON1 Status Protocol with Non-OP Substrates

Since PON1 status appears to be an important risk factor for OP exposure as well as for a number of diseases, we examined rates of hydrolysis of more than 70 substrates under different conditions of salt concentration and pH to develop a high throughput PON1 status protocol that did not make use of the highly toxic OP substrates. Figure 2 shows a comparison of the DZOase vs. POase protocol for determining PON1 status and a new PON1 status protocol where rates of phenyl acetate hydrolysis at high salt are plotted against rates of 4-(chloromethyl) phenyl acetate (CMPA) in buffer alone (Richter et al. 2008,2009). Both of these protocols resolve all three $PON1_{192}$ phenotypes; however the new assay with non-OP substrates is more suitable for laboratories that are not equipped for using highly toxic compounds.

Since many assay protocols have been used by different laboratories over the years, we determined conversion factors that will allow inter-conversion of rates of hydrolysis of one substrate to another for each of the three $PON1_{192}$ phenotypes (Richter et al. 2009) (Table 1). Also, we described how to determine physiologically relevant rates of in vivo hydrolysis of CPO and DZO. This new assay should encourage epidemiologists to measure the parameters that are important in relating genetic variability of PON1 to risk of disease or exposure.

We have shown previously that discrepancies between $PON1_{192}$ SNP analysis and the functional PON1 status analysis can reveal mutations in the *PON1* gene that can be characterized by sequencing the entire *PON1* gene (Jarvik et al. 2003). The effects of polymorphisms in the 3′-untranslated region of the *PON1* gene have yet to be characterized. While there is still much to learn about the effects of *PON1* SNPs on expression, it will be important to couple such studies with the functional PON1 status analysis. Although the effects of some environmental influences on plasma PON1 levels are known (reviewed in Costa et al. 2005), the effects of epigenetic modifications on PON1 expression are yet to be explored.

Acknowledgments

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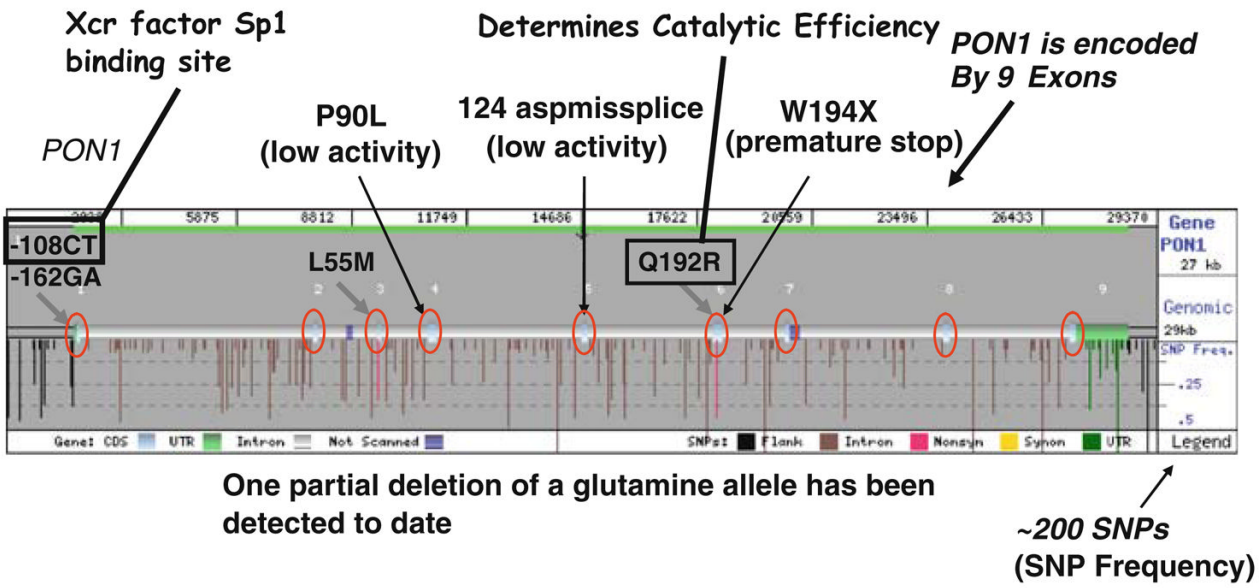

SeattleSNPs http://pga.gs.washington.edu/ ; Furlong et al. 2008

Fig. 1.

The human *PON1* gene with known polymorphisms and their frequencies. The 5′ end of the gene is on the *left*. (Seattle SNPs, http://pga.gs.washington.edu/)

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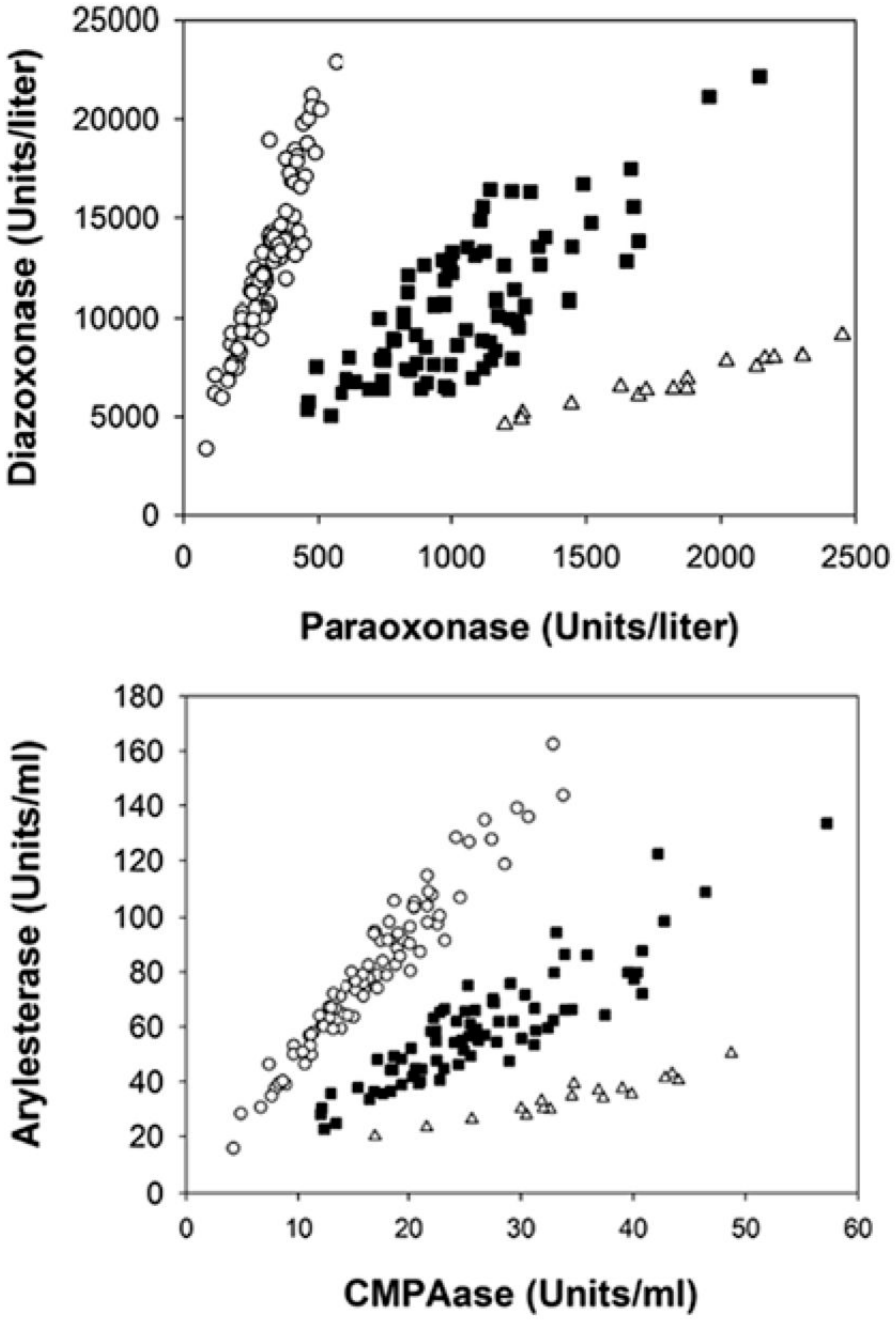

Fig. 2.

Comparison of the two protocols for determining PON1 status. (**a**) Assays using the highly toxic OP substrates DZO and PO; and (**b**) assays using the non-OP substrates phenyl acetate and CMPA. The 183 plasma samples included 86 $PON1_{Q192}$ homozygotes, 79 heterozygotes and 18 $PON1_{R192}$ homozygotes with genotypes verified by PCR. Reproduced from Richter et al. (2009) with permission

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Table 1

Conversion factors for rates of substrate hydrolysis

Phenotype	Conversion factors	r^2 [a]
QQ	$AREase_{HS}$[b] (U/ml) × 172 = $DZOase_{phys}$ (U/L)[c]	0.93
QR	$AREase_{HS}$ (U/ml) × 204 = $DZOase_{phys}$ (U/L)	0.82
RR	$AREase_{HS}$ (U/ml) × 286 = $DZOase_{phys}$ (U/L)	0.87
QQ	$AREase_{HS}$ (U/ml) × 69 = $CPOase_{phys}$[d] (U/L)	0.87
QR	$AREase_{HS}$ (U/ml) × 103 = $CPOase_{phys}$ (U/L)	0.88
RR	$AREase_{HS}$ (U/ml) × 189 = $CPOase_{phys}$ (U/L)	0.89
QQ	$AREase_{LS}$[e] (U/ml) × 110 = $DZOase_{phys}$ (U/L)	0.84
QR	$AREase_{LS}$ (U/ml) × 100 = $DZOase_{phys}$ (U/L)	0.72
RR	$AREase_{LS}$ (U/ml) × 83 = $DZOase_{phys}$ (U/L)	0.93
QQ	$AREase_{LS}$ (U/ml) × 45 = $CPOase_{phys}$ (U/L)	0.73
QR	$AREase_{LS}$ (U/ml) × 50 = $CPOase_{phys}$ (U/L)	0.84
RR	$AREase_{LS}$ (U/ml) × 55 = $CPOase_{phys}$ (U/L)	0.92
QQ	$AREase_{HS}$ (U/ml) × 3.8 = POase (U/L)	0.75
QR	$AREase_{HS}$ (U/ml) × 15.9 = POase (U/L)	0.50
RR	$AREase_{HS}$ (U/ml) × 47.6 = POase (U/L)	0.90
QQ[f]	$AREase_{HS}$ (U/ml) × 1.6 = $AREase_{LS}$ (U/ml)	0.85
QR[f]	$AREase_{HS}$ (U/ml) × 2.0 = $AREase_{LS}$ (U/ml)	0.66
RR[f]	$AREase_{HS}$ (U/ml) × 3.5 = $AREase_{LS}$ (U/ml)	0.83
QQ	$DZOase_{phys}$ (U/L) × 1.08 = $DZOase_{HS}$[g] (U/L)	0.90
QR	$DZOase_{phys}$ (U/L) × 1.01 = $DZOase_{HS}$ (U/L)	0.91
RR	$DZOase_{phys}$ (U/L) × 0.84 = $DZOase_{HS}$ (U/L)	0.87

[a] Correlation coefficient squared

[b] $AREase_{HS}$ = Arylesterase activity measured in buffer and 2 M NaCl

[c] $DZOase_{phys}$ = Diazoxonase activity measured under physiological conditions

[d] $CPOase_{phys}$ = Chlorpyrifos oxonase activity measured under physiological conditions

[e] $AREase_{LS}$ = Arylesterase activity measured in buffer

[f] From Richter et al. (2008)

[g] $DZOase_{HS}$ = Diazoxonase activity measured at 2 M NaCl, pH 8.5 (reproduced from Richter et al. 2009, with permission)