Role of Paraoxonase (PON1) Status in Pesticide Sensitivity: Genetic and Temporal Determinants

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Abstract

Individual differences in detoxication capacities for specific organophosphorous (OP) compounds are due largely to differences in catalytic efficiency or abundance of the HDL-associated enzyme, paraoxonase (PON1). First, we provide evidence that children less than 2 years of age represent a particularly susceptible population for OP exposure due to low abundance of PON1 and variable onset of plasma PON1 activity. Second, we describe studies examining the neurotoxic effects of chronic, low-level OP pesticide exposure in mice. PON1 knockout (PON1−/−) and wild-type mice were exposed chronically (PN4 to PN21) to low levels of chlorpyrifos oxon (CPO). Endpoints included cholinesterase activity, histopathology, gene expression, and behavior. Even at PN4, when PON1 levels were low in wild-type mice, PON1−/− mice were more sensitive to inhibition of brain cholinesterase by CPO. At PN22, and persisting as long as 4 months, chronic developmental exposure to 0.18 mg/kg/d or 0.25 mg/kg/d CPO resulted in perinuclear vacuolization of cells in a discrete area of the neocortex and irregular distribution of neurons in the cortical plate, with an increase in the number of affected cells at 0.25 mg/kg/d. Third, we describe a transgenic mouse model in which human transgenes encoding either hPON1Q192 or hPON1R192 were expressed at equal levels in place of mouse PON1. The developmental onset of expression followed the mouse time course and was identical for the two transgenes, allowing these mice to be used to assess the importance of the Q192R polymorphism during development. Adult mice expressing hPON1R192 were significantly more resistant than hPON1Q192 mice to CPO toxicity. Our studies indicate that children less than 2 years old, especially those homozygous for PON1Q192, would be predicted to be particularly susceptible to CPO toxicity.

Keywords: Paraoxonase; PON1; Organophosphorous compound; Polymorphism

INTRODUCTION

This review focuses on specific genetic and temporal factors that influence sensitivity to the commonly used insecticides chlorpyrifos (Dursban®) and diazinon, with a focus on the high-density lipoprotein (HDL)-associated enzyme paraoxonase (PON1). PON1 exhibits considerable variation among individuals due to a coding region polymorphism that alters its activity and to differences among individuals in plasma enzyme levels, which also vary temporally during development. Genetic and temporal variability in this enzyme affects not only sensitivity to pesticides, but also risk for vascular disease and pharmacokinetics of drug
metabolism, due to its ability to hydrolyze substrates other than the organophosphorous (OP) insecticides.

EARLY STUDIES

Mazur (1946) first described enzyme activity in plasma that was capable of hydrolyzing OP compounds. Careful and insightful studies by Aldridge (1953a, 1953b) described an esterase that hydrolyzed certain aromatic esters and OP compounds like paraoxon, the active metabolite of parathion. Aldridge designated this esterase as A-esterase. In addition, Aldridge described a B-esterase that, in contrast to A-esterase, was inhibited by organophosphates. His hypothesis that the same enzyme, A-esterase, hydrolyzed both phenylacetate and paraoxon was proven conclusively only recently, with the demonstration that recombinant paraoxonase/arylesterase (PON1, EC 3.1.8.1) catalyzed both activities (Sørenson et al., 1995). The plasma enzyme paraoxonase (PON1) is the A-esterase described by Aldridge, whereas B-esterase activity is represented by the plasma cholinesterases. A survey of the plasma levels of A-esterase activity in different species showed wide variation among the species surveyed (Brealey et al., 1980; Costa et al., 1987). Species exhibiting higher levels of plasma A-esterase activity were also more resistant to the toxicity of OP compounds than were species with lower enzyme levels. This observation suggested that inter-species differences in sensitivity to OP compounds are due largely to variability in their plasma levels of A-esterase. In humans, plasma paraoxonase levels vary by at least 13-fold (Davies et al., 1996). In human populations, early studies examining paraoxonase levels revealed a polymorphic distribution of paraoxonase activity, with either bi- or trimodal distributions reported, depending on the particular assay used (Eckerson et al., 1983; Geldmacher von Mallinckrodt and Diepgen, 1988; Mueller et al., 1983). While the enzyme responsible for the activity had not yet been identified, differences in gene frequencies among different ethnic groups were easily seen with even a simple assay. Over the years, the assays used to investigate the paraoxonase activity polymorphism were improved (Ortigoza-Ferado et al., 1984). A major advance was made by Eckerson et al. (1983), who developed a two-substrate assay where the rate of paraoxon hydrolysis for each plasma sample was measured and plotted against its corresponding rate of phenylacetate hydrolysis. This approach clearly separated the low metabolizers from the high metabolizers, and came close to resolving the presumed heterozygotes from the high metabolizers. It became clear from this study that even within a genetic class, e.g., within the low metabolizers, there was wide variability in enzyme activity levels in plasma. A refinement of this two-substrate approach for assessment of PON1 activity, that uses diazoxon instead of phenylacetate (Richter and Furlong, 1999), is discussed further.

MOLECULAR BASIS OF THE PON1 POLYMORPHISM

The molecular basis of the PON1 activity polymorphism was not elucidated until the human PON1 cDNA was isolated and sequenced (Adkins et al., 1993; Hassett et al., 1991). Two interesting pieces of information came out of the initial sequencing effort. First, it became evident that PON1 retained its signal sequence, having only the initiator methionine removed during secretion. Second, two research groups reported independently that variability in the amino acid present at position 192 [glutamine (Q) or arginine (R)], due to a single nucleotide polymorphism, was responsible for the PON1 activity polymorphism (Adkins et al., 1993; Humbert et al., 1993). The consequences of the PON1Q192R polymorphism for OP detoxication are discussed in more detail further. Another polymorphism was identified that resulted in an amino acid substitution [leucine (L)/methionine (M)] at codon 55. Some reports have noted an association between the PON1M55 alloform and low PON1 activity (Blatter Garin et al., 1997; Brophy et al., 2001b; Mackness et al., 1998). It should be noted that initially there was a discrepancy in the literature in the nomenclature of these polymorphisms. Whereas some published reports numbered the amino acids from the first residue of the mature protein (Adkins et al., 1993), others numbered the amino acids from the initiator methionine (Hassett et al., 1991; Humbert et al., 1993). Thus, early papers that report the position of the polymorphisms as 191 Q/R and 54 L/M are referring to the same polymorphisms reported by others to be at positions 192 Q/R and 55 L/M. This issue was finally settled during a conference on OP hydrolases in Dubrovnik, Croatia, where it was decided to number the amino acids beginning with the initiator methionine (i.e., 192 Q/R and 55 L/M) (La Du et al., 1999). The nomenclature for the many new polymorphisms revealed recently (Jarvik et al., 2003) needs also to be standardized. For example, the SP1 binding site polymorphism is reported alternatively to be at nucleotide position −107 C/T or −108 C/T.
OTHER SUBSTRATES AFFECTED BY THE PON1Q192R POLYMORPHISM

Davies et al. (1996) extended the two-substrate approach to a number of other OP compounds, including chlorpyrifos oxon, diazoxon, soman and sarin. Interestingly, the effect of the activity polymorphism was reversed for soman and sarin. While PON1R192 hydrolyzed paraoxon at a faster rate than did PON1Q192, the opposite was true for soman and sarin, under the conditions used for the in vitro assays. Davies et al. (1996) also reported that PON1R192 hydrolyzed diazoxon less well than did PON1Q192. As described further, this observation was the result of the supra-physiological salt concentration (2 M) used in the in vitro assays. At physiological salt concentrations, the two alloforms had equivalent catalytic efficiencies of hydrolysis of diazoxon (Li et al., 2000).

Recently, rates of hydrolysis of a number of drugs that contain lactone-rings or cyclic carbonates have been examined (Biggadike et al., 2000; Billecke et al., 2000). The PON1Q192R polymorphism was shown to influence the rates of some of the drugs/prodrugs examined. Activation of the antibacterial prodrug, prulifloxacin to the active quinolone antibiotic NM394 by PON1 occurred at a higher rate with the PON1R192 alloform, which also hydrolyzed thiolactones more readily (Billecke et al., 2000; Tougou et al., 1998). Inactivation of glucocorticoid γ-lactones by PON1 confines their mode of action to the sites of application, preventing serious systemic side effects (Biggadike et al., 2000). PON3 appears to be responsible for the hydrolysis of statin lactones and the diuretic spironolactone (Draganov and La Du, 2004).

In contrast, the PON1Q192 alloform was more efficient at reducing lipid-peroxides in homogenates of human atherosclerotic lesions (Aviram et al., 2000). A considerable amount of research still needs to be carried out to understand the physiological consequences of both variation in PON1 levels and the position 192 Q/R polymorphism with respect to all of the many different activities catalyzed by PON1.

PON1 STATUS AND EPIDEMIOLOGICAL STUDIES

Two-substrate analysis using the substrate pair paraoxon and diazoxon turned out to provide a complete resolution of the three PON1 phenotypes and provided a very accurate means of determining PON1Q192R functional genotype (Richter and Furlong, 1999; Fig. 1). This analysis, like the earlier analysis reported by Eckerson et al. (1983), clearly pointed out the large inter-individual variability in plasma PON1 levels. Fundamental biochemical principals dictate that the rate of metabolism of a given toxin should be proportional to the concentration of the enzyme involved in its detoxication. Thus, it should have been clear early on that plasma levels of PON1 would be important for epidemiological studies examining the relationship of PON1 genetics to risk of disease or exposure. Despite early knowledge that both PON1Q192R genotype and PON1 levels are important in governing rates of toxin metabolism, many epidemiological studies have been carried out where only single nucleotide polymorphisms have been examined (reviewed in Brophy et al., 2002). Since very few of these studies included a determination of PON1 levels (ignoring the significant contribution that differences in plasma enzyme levels play in determining risk), they are of limited value in estimating disease association with PON1. For epidemiological studies where the PON1Q192R polymorphism is suspected of affecting the metabolism of the compound in question (e.g., oxidized lipids, OP compounds, or drugs), PON1 levels should be measured in addition to ascertaining genotype at PON1Q192R. The combination of determining genotype at PON1Q192R and measuring PON1 levels in plasma has been designated as determining an individual’s “PON1 status” (Li et al., 1993; Richter and Furlong, 1999).
A recent study examined how well PCR genotyping corresponded with PON1 status, as measured using the two-substrate functional assay (Jarvik et al., 2003). A number of individuals in the study had discrepancies between their PON1Q192R genotype and their phenotypic assignment in the functional assay (Fig. 1). Only after sequencing the entire PON1 genes from these individuals was it discovered that a number of them had only one functional PON1 allele, due to splicing or mutation including premature truncation defects in the other allele. Since PON1Q192R genotyping could not pick up these defects, it provided an inaccurate estimate of PON1 status in these individuals, and would give an unreliable estimate of that individual’s risk for sensitivity to OP compounds or for vascular disease. These examples provide yet another reason for carrying out functional analyses of PON1 activity using high-throughput enzyme assays. All of the 192 known single nucleotide polymorphisms in PON1 could be identified in as individual and still not provide the information gleaned from the two-substrate determination of PON1 status.

ROLE OF PON1 IN OP DETOXICATION

While it was assumed that high levels of PON1 would protect against exposure to specific OP compounds, only a single experiment that directly addressed this question had been reported prior to 1990. Main (1956) reported that injection of partially purified PON1 into rats increased their resistance to paraoxon. This observation was confirmed and extended through a series of experiments begun in our laboratory in 1990. Injection of purified rabbit paraoxonase into rats increased their resistance to paraoxon exposure (Costa et al., 1990). Following this second demonstration that injected PON1 could increase resistance to paraoxon, we switched to a mouse model for two major reasons. First, many more experiments could be carried out with a given quantity of purified PON1. Second, it was clear that genetics were much further advanced in mice than rats, with the capability existing for engineering mice with altered PON1 levels.

Injection of purified rabbit PON1 into mice 4 h prior to exposure dramatically increased their resistance to chlorpyrifos oxon (Li et al., 1993). An increase in resistance to the parent compound, chlorpyrifos, was also observed (Li et al., 1995). These experiments demonstrated clearly that high levels of plasma paraoxonase could protect against exposure to chlorpyrifos oxon or chlorpyrifos. Protection was also observed when purified rabbit PON1 was injected post-exposure or 24 h prior to exposure, indicating that administration of purified or recombinant PON1 would be useful for ameliorating or even preventing adverse consequences of exposure to OP compounds.

Whereas higher PON1 levels were demonstrated clearly to be protective, determining whether low levels of PON1 would result in greater sensitivity was not possible until the development of PON1 knockout mice, generated by Drs. Jake Lusis, Diana Shih and co-workers (Shih et al., 1998). Knocking out the mouse PON1 gene resulted in a dramatic increase in sensitivity to chlorpyrifos oxon exposure and a modest increase in sensitivity to chlorpyrifos exposure, as assessed by measuring brain cholinesterase inhibition. Dermal exposures to levels of chlorpyrifos oxon that produced no symptoms of cholinergic effects and minimal inhibition of brain cholinesterase in wild-type mice were unexpectedly lethal to the PON1 null mice. Similar results were observed when the knockout mice were exposed to diazoxon (Li et al., 2000). Dermal exposure to 2 or 4 mg/kg diazoxon produced no measurable effect in wild-type mice, but was lethal to the PON1 knockout mice, and exposure to 1 mg/kg diazoxon had significant adverse effects in the knockout mice without measurably affecting the wild-type mice. Hemizygous mice, with only one PON1 allele, exhibited intermediate sensitivity. Exposure of the PON1 knockout mice to paraoxon, however, produced an unexpected and initially puzzling result. They were not any more sensitive than wild-type mice to paraoxon exposure.

Further experiments demonstrated that resistance of the PON1 knockout mice to diazoxon was restored by injection of purified PON1R192 or PON1Q192 alloforms, with either alloform providing equivalent protection (Li et al., 2000). Resistance to chlorpyrifos oxon was also restored; however, the PON1R192 alloform provided significantly better protection that did the PON1Q192 alloform. Neither alloform provided protection against paraoxon exposure.

CATALYTIC EFFICIENCY IS THE KEY TO PROTECTION

All of these observations were explained by an examination of the catalytic efficiencies of the PON1Q192 and PON1R192 alloforms for the hydrolysis of each of the OP compounds (Li et al., 2000). Both alloforms hydrolyzed diazoxon with equivalent cata-
lytic efficiencies \(V_{\text{max}}/K_m = 77\). Even though PON1_{R192} hydrolyzed paraoxon with a nine-fold greater efficiency \(V_{\text{max}}/K_m = 6.27\) than did PON1_{Q192} \(V_{\text{max}}/K_m = 0.71\), the catalytic efficiency was not sufficient to provide protection against exposure to paraoxon in vivo. The better protection provided by PON1_{R192} against chlorpyrifos oxon exposure in vivo was explained by its catalytic efficiency \(V_{\text{max}}/K_m = 256\) being higher than that of PON1_{Q192} \(V_{\text{max}}/K_m = 150\). Pond et al. (1995) had predicted that PON1 would protect against exposure to chlorpyrifos oxon, but would not protect against exposure to paraoxon, based on in vivo rates of hydrolysis of these two compounds.

More recent studies with mice expressing either human PON1_{Q192R} alloform (on a PON1 knockout background) are consistent with the results described earlier. Mice expressing human PON1_{Q192} were nearly as sensitive as the PON1 knockout mice to chlorpyrifos oxon exposures, while mice expressing human PON1_{R192} exhibited significantly greater resistance to these exposures (unpublished results). This observation is significant in that PON1_{Q192} homozygotes comprise as much as 50% of populations of Northern European origin (Brophy et al., 2002).

These observations provide important guidelines for engineering recombinant PON1 for therapeutic applications. For PON1 to protect against toxicity of a given OP compound (or other PON1 substrate), the catalytic efficiency in vivo must be sufficiently high, or no protection will be provided by PON1, regardless of whether the source of PON1 is endogenous or injected (purified native or recombinant).

**INTERPRETING THE CONSEQUENCES OF PON1 GENETIC VARIABILITY**

While there was some protection afforded by PON1 against the respective parent compounds, the protective effects of PON1 were most striking with the oxon forms of chlorpyrifos and diazinon. The parent OP compound is converted to its more toxic oxon form in the liver, by cytochrome P450-mediated oxidative desulfuration, and the oxon form serves as the direct substrate for PON1. However, direct exposure to the oxon forms of the OPs in the field is also possible. This caused us to revisit a survey that we had published some years ago that summarized the percentages of oxon found in foliar residues (Yuknavage et al., 1997), which varied from a few percent to as much as 97% of the OP residue (Table 1). While values were not reported for chlorpyrifos, a California EPA survey of the oxon content of drift residues during spraying of chlorpyrifos in Tulare County noted significant oxon content in most of the samples analyzed (California Air Resources Board, 1998). Since chlorpyrifos oxon inhibits acetylcholinesterase at least 1000 times more rapidly than chlorpyrifos (Huff et al., 1994), even a small percentage of oxon content is important with respect to an individual’s PON1 status.

**REGULATION OF PON1 EXPRESSION**

The wide variability in plasma PON1 levels among individuals is due at least in part to polymorphisms that have been identified in the 5′ regulatory region of PON1. Our research group and two others have exam-

<table>
<thead>
<tr>
<th>Study</th>
<th>Pesticide (units)</th>
<th>Oxon a</th>
<th>Thioate</th>
<th>Total OP</th>
<th>Oxon (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ralls et al. (1966)</td>
<td>Diazinon (ppm) b</td>
<td>0.05</td>
<td>0.25</td>
<td>0.3</td>
<td>17</td>
</tr>
<tr>
<td>Kansouh and Hopkins (1968)</td>
<td>Diazinon e</td>
<td>ND d</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>Wolfe et al. (1975)</td>
<td>Parathion (ng/cm²) b</td>
<td>8</td>
<td>106</td>
<td>114</td>
<td>7</td>
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<tr>
<td>Nigg et al. (1977)</td>
<td>Ethion (ng/cm²) b</td>
<td>42</td>
<td>285</td>
<td>327</td>
<td>13</td>
</tr>
<tr>
<td>Spear et al. (1977a)</td>
<td>Parathion (ng/cm²) b</td>
<td>84</td>
<td>29</td>
<td>113</td>
<td>74</td>
</tr>
<tr>
<td>Spear et al. (1977b)</td>
<td>Parathion (µg) e</td>
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<td>39</td>
<td>184</td>
<td>79</td>
</tr>
<tr>
<td>Costello et al. (1989)</td>
<td>Parathion (ng/cm²) b</td>
<td>229</td>
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<td>237</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Malathion (µg) e</td>
<td>659</td>
<td>2301</td>
<td>2960</td>
<td>22</td>
</tr>
</tbody>
</table>

Table modified from Table 1 of Yuknavage et al. (1997).

a Based on the highest value reported in study.
b Foliar residue measurement.
c Units or values not given in study.
d ND: none detected.
e Dermal monitoring measurement.
ined the effects of some of these polymorphisms on plasma PON1 levels (Brophy et al., 2001a, 2001b, 2002; Leviev and James, 2000; Suehiro et al., 2000). All three groups concluded that the C-108T polymorphism had the greatest effect on plasma PON1 levels, with the C-108 allele producing about twice the level of plasma PON1 as the T-108 allele. The C-108T polymorphism is in the middle of a consensus sequence (GGCGGG) for binding of the transcription factor, Sp1. This site is also important for upregulation of PON1 by statins, and perhaps ethanol (Rao et al., 2003). Upregulation of PON1 expression by simvastatin involves an additional sequence in the 5′ regulatory region that represents a binding site for another transcription factor, sterol regulatory element binding protein 2 (SREBP-2; Deakin et al., 2003). Deakin et al. (2003) also reported an interaction between Sp1 and SREBP-2 in binding to the PON1 promoter.

Recently, the laboratory of Deborah Nickerson sequenced the entire PON1 genes from 47 individuals, and identified more than 160 new polymorphisms, including 1 novel cSNP, 8 new SNPs in the 5′ regulatory region, and 12 new SNPs in the 3′ UTR (Jarvik et al., 2003). Among the new genetic variations observed was a W194X mutation which prompted us to reexamine DNA from plasma samples of individuals where there had been a discrepancy between their PON1Q192R genotyping results and their PON1 status as determined by the two-substrate assay. One individual was identified among those samples as having the W194X mutation (Fig. 1; Jarvik et al., 2003). Re-sequencing the PON1 genes of other individuals who had a discrepancy between their PON1Q192R allele and the PON1 promoter revealed three additional mutations, an amino acid substitution at L90P, a probable deletion of part of the PON1Q192 allele and a mutation in an Asp codon that affected splicing (Jarvik et al., 2003).

Recent exploration of other polymorphisms that affect the efficiency of RNA splicing (Faustino and Cooper, 2003) suggest that the newly discovered polymorphisms found in introns may well occur in splice enhancer sequences and thus result in subtle, or even significant, changes in PON1 expression levels. Determining which ones of these many intronic polymorphisms affect PON1 expression will be a difficult but most likely rewarding effort.

DEVELOPMENTAL REGULATION OF PON1 EXPRESSION

The observations described earlier provided convincing evidence that PON1 plays a major role in determining sensitivity to chlorpyrifos/chlorpyrifos oxon and diazinon/diazoxon exposures and prompted us to re-examine one of our earlier observations of low PON1 levels in newborns (Mueller et al., 1983). It became of interest to define the time course of appearance of PON1 activity in the plasma of newborns. We found that individual infants approached plateau levels of PON1 in their sera at different times, varying from 6 to 15 or more months (Cole et al., 2003). It is reasonable to assume that PON1 levels are even lower in fetuses, leading to concern about exposure of the fetuses of mothers with low PON1 status to specific compounds.

Another related set of experiments examined the time course of appearance of the human PON1 alloforms in mice expressing hPON1Q192 and hPON1R192 on the PON1 null background (Cole et al., 2003). Interestingly, these studies showed that expression of the human PON1 transgenes under control of the human PON1 5′ regulatory sequences followed the same time course of appearance seen with mouse PON1; i.e., the levels of the two hPON1 alloforms peaked around PND 21, whereas in humans, as noted earlier, mature levels of expression are reached at 6–15 months. These experiments indicated that the regulatory elements controlling the developmental expression of PON1 are mostly conserved between mouse and man.

There have been a number of reports related to the influences of diet and environmental factors such as smoking on PON1 expression or activity. The reader is referred to a recent review of this topic for more information on this subject (Costa et al., submitted for publication).

EPIDEMIOLOGICAL STUDIES AND PON1 LEVELS

To date, more than 100 studies have been carried out with the aim of determining whether there is a relationship between genetic variations in PON1 and risk for specific diseases including vascular disease, vascular disease among diabetics, Alzheimer’s disease and Parkinson’s disease (reviewed in Brophy et al., 2002). The vast majority of these studies have considered only specific single nucleotide polymorphisms (SNPs) or haplotype associations, ignoring the importance of variability in PON1 levels. This discussion is a plea for investigators to make use of the simple high-throughput determination of PON1 status via the two-substrate analysis that was used to generate the
data in Fig. 1. Many factors go into establishing an individual’s PON1 status, but only some of these are currently known. Even if all known PON1 SNPs were analyzed in an individual, it would not be possible to predict the circulating levels of PON1 in their plasma, whereas the enzymatic determination of PON1 status using the two-substrate assay provides the functional PON1Q192R phenotype as well as the circulating levels of PON1, the two factors involved in protecting an individual against toxic lipids and OP compounds. The same considerations bear on the questions of genetic variability of PON1 and pharmacokinetics of drug metabolism through the PON1 pathway.

This discussion can also be viewed as a plea to the epidemiological community to avoid analyzing only PON1 SNP data. It will be more informative to analyze PON1 status for each individual in the study, and to consider carefully not only the effects of the PON1Q192R polymorphism, but also the relative contribution of other metabolic pathways that might be involved in metabolizing the toxin or drug of interest. The examples cited earlier include cases where both PON1Q192R genotype and PON1 levels are important, such as chlorpyrifos oxon exposure or vascular disease, and cases where PON1 level is the important factor governing sensitivity, such as diazoxon exposure. The same will hold for many other substrates of this promiscuous enzyme.

**SUMMARY**

PON1 status plays an important role in protecting against exposures to diazinon and chlorpyrifos, particularly to the oxon residues present in these exposures. It is also clear that the developmental regulation of expression of PON1 is an important component of this picture. The most important conclusion to come from our studies is that to understand the role that PON1 plays in an individual’s sensitivity or resistance to a given exposure or in the pharmacokinetic disposition of a specific drug, it is important to know both the levels of PON1 and in some cases the phenotype of the position 192 codon. These two important pieces of information are readily extracted from a two-substrate determination of the individual’s plasma PON1 status.

**FUTURE DIRECTIONS**

The studies performed to date have provided clear evidence that PON1 plays a major role in detoxication of the oxon forms of diazinon and chlorpyrifos oxon. It is also clear from these studies that other pathways are important for detoxifying the parent compounds, as well as other OP compounds such as parathion and paraoxon. The availability of the PON1 knockout mice provides an excellent system in which to investigate the contributions of these other pathways without the interference of PON1. For example, with these animals it will be possible to examine the contribution of the carboxylesterases, cholinesterases and cytochromes P450 in the detoxica-

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