Paraoxonase 1 (PON1) Status and Risk of Insecticide Exposure

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Paraoxonase 1 (PON1) is an HDL associated enzyme that catalyzes a number of different reactions including the hydrolysis of the toxic oxon metabolites of the insecticides diazinon and chlorpyrifos. PON1 has also been implicated in the detoxication of oxidized lipids and the metabolism of a number of drugs, activating some, while inactivating others. There are two common PON1 coding region polymorphisms (L55M and Q192R). The latter determines the catalytic efficiency of hydrolysis of a number of substrates including chlorpyrifos oxon, but not diazoxon.

Evidence for the physiological importance of PON1 in modulating exposures to these two insecticides comes from several different studies. Early studies noted that species with high levels of PON1 were much more resistant to certain organophosphorus (OP) insecticides than were species with low levels. Another early study by Main demonstrated that injected rabbit paraoxonase protected rats from paraoxon toxicity. Our research group began the development of a mouse model system for examining the importance of PON1 in the detoxication of OP insecticides.

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The first sets of experiments demonstrated that injecting purified rabbit PON1 into rats or mice significantly increased resistance to chlorpyrifos and chlorpyrifos oxon, thus demonstrating that high PON1 levels were protective against exposure. They also showed that the protection afforded to chlorpyrifos oxon exposure was significantly better than provided for chlorpyrifos exposure. A survey of reported oxon values in foliar residues indicated that there are oxon residues in most exposures.

The consequences of low levels of plasma PON1 were examined in PON1 knockout mice generated by Lusis, Shih and co-workers at UCLA. These mice were found to be highly sensitive to exposures to either diazoxon or chlorpyrifos oxon, but surprisingly not to paraoxon. Examination of the catalytic efficiencies of PON1 for hydrolysis of each of these oxons provided a clear explanation for these observations. While $PON1_{R192}$ hydrolyzed paraoxon ninetimes more efficiently than $PON1_{Q192}$ (6.27 vs. 0.71), the efficiency was not sufficient to provide protection against a paraoxon exposure. On the other hand, the catalytic efficiencies for hydrolysis of diazoxon by the two PON1-192 alloforms were equivalent and ten-times more efficient (~77) than for hydrolysis of paraoxon by $PON1_{R192}$. $PON1_{R192}$ had significantly greater catalytic efficiency (250) for hydrolyzing chlorpyrifos oxon than did PON1Q192 (150), and both were significantly higher than the efficiency of $PON1_{R192}$ for hydrolyzing paraoxon. Injection of the two purified human PON1-192 alloforms into PON1 knockout mice reconstituted the PON1 null mice with either of the human alloforms. These animals were challenged 4 h later with paraoxon, diazoxon, or chlorpyrifos oxon. The

results were consistent with the determined catalytic efficiencies of substrate hydrolysis. No protection was observed for paraoxon exposure, either allform protected equally as well against diazoxon exposure and $PON1_{R192}$ protected significantly better than $PON1_{Q192}$ against chlorpyrifos oxon exposure. Thus, for risk of diazoxon exposure, it is important to know only an individual's plasma PON1 levels, for chlorpyrifos oxon exposure it is important to know both plasma PON1 levels and position 192 genotype.

A two-substrate assay/analysis was developed that provides both PON1 phenotype and functional genotype. Plotting the rates of diazoxon hydrolysis (at high salt) vs. paraoxon hydrolysis by plasma from a given population breaks the data into three clear groups, individuals homozygous for $PON1_{Q192}$, heterozygotes, and individuals homozygous for $PON1_{R192}$ (Figure 1). In addition to providing the functional position 192 alloform(s), this analysis also provides the levels of the individuals' plasma PON1 which are at least as important, if not more so, than the position 192 amino acid (Q or R). This functional analysis has been referred to as PON1 status, i.e., it provides both functional position 192 genotype as well as phenotype. It also provides an excellent means of examining risk associated with an individual's PON1 status.

Another important factor to consider in analyzing an individual's PON1 status for risk analysis is age. PON1 levels in plasma are quite low at birth and do not reach mature levels until between 6 and 24 months of age, depending on the individual. Thus, one concern is exposure of a fetus whose mother who has very low PON1 status.

In summary, in examining PON1 genetic variability as a risk factor for exposures or disease (e.g. vascular disease), it is important to carry out the two-substrate, high throughput assay rather than SNP analysis which provides inadequate data for estimating risk.

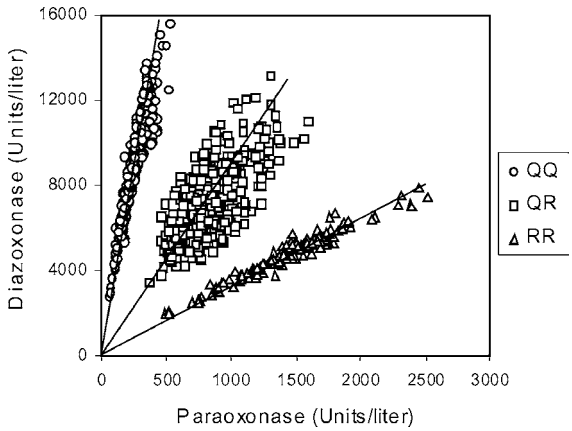

FIGURE 1. Determination of PON1 status. Each data point represents the initial rates of hydrolysis of diazoxon and paraoxon by citrate plasma from a single individual. Note that this two-dimensional analysis divides the population clearly into three groups, individuals homozygous for $PON1_{Q192}$, heterozygotes, and individuals homozygous for $PON1_{R192}$. In addition to providing the functional position 192 genotype, the levels of PON1 in the individuals' plasma are also revealed. Note also that the data points for both classes of homozygotes fall tightly along the trend lines, while heterozygote data points vary considerably from the trend line. We interpret this observation to indicate that the individuals on one side or the other of the trend line are producing more PON1 from one of their PON1 alleles (Q192 or R192) (*Current Protocols in Toxicology* 4.12, 2004). These data clearly indicate that SNP analysis of PON1 (even analysis of all ~200 SNPs) will not provide the information available from this high-throughput two-substrate analysis. Individuals with the same PON192 genotype will vary from 5- to 13-fold in their ability to detoxify specific OP compounds or other substrates.