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CDC/ATSDR TOXICOLOGY REPORT

Serum Paraoxonase-1 (PON-1) Genotype and Exposure to Organophosphorous Insectides—Is There a High-Risk Population?

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Abstract The Health Studies Branch (HSB) is responsible for responding to domestic and international requests for assistance with suspected and known environmental-associated public health threats as well as pursuing original environmental research. The HSB employs personnel with a wide variety of educational backgrounds and professional training including epidemiology, medicine, toxicology, statistics, and other environmental public health-related disciplines. This wide range of expertise is necessary to address the broad scope of potential environmental health threats. HSB scientists conduct studies on environmental exposures. Recent examples include the following: mercury exposure in children living in large urban areas, exposure to brevetoxins and microcystins arising from harmful algal blooms, and occupational exposures to pesticides. This article will present a brief description of an ongoing study of insecticide exposure and paraoxonase-1 (PON-1) genotype in banana plantation workers in Chinandega, Nicaragua. We will then discuss the enzyme PON-1 and its potential role in organophosphate insecticide metabolism and toxicity.

Keywords PON-1 · Organophosphate

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Current CDC Activity: PON-1 and Chlorpyrifos Exposure Among Agricultural Workers

Paraoxonase-1, a serum enzyme closely associated with high-density lipoprotein (HDL), plays a role in lipid and OP metabolism. Various mutations in the PON-1 gene are known to determine the efficacy of OP metabolism by PON-1. Animal studies show that PON-1 genotype and the rate of OP metabolism play a major role in determining resistance to the acute toxicity of OPs [4]. However, the

relationship between an individual's PON-1 genotype and its interaction with OPs remains unclear in human populations and requires further research.

In order to investigate the relationship between OPs and individual PON-1 genotype, medical toxicologists and epidemiologists from HSB are conducting a cohort study composed of 186 agricultural workers at three banana plantations in Chinandega, Nicaragua that began in May 2009. The objective of the study is to determine whether a select group of agricultural workers are at increased risk of health effects due to chlorpyrifos (an OP insecticide commonly applied to crops in Nicaragua) exposure and determine whether PON-1 phenotype had an impact on subsequent health effects. The study group included two populations: workers exposed to chlorpyrifos and those who work at the plantation, but are not directly exposed to chlopyrifos. We administered a health-screening questionnaire, which asked about workers' cardiovascular risk factors, preexisting neurological diseases, dietary and medical history, as well as basic demographic information. A physician on the team also examined each participant for abnormalities consistent with chlorpyrifos toxicity. We collected urine samples to test for the presence of urinary metabolites of chlorpyrifos and blood samples to test for PON-1 genotype, PON-1 phenotype, cholesterol levels, and plasma and erythrocyte cholinesterase levels. We will analyze these data to look for correlations between PON-1 genotype and phenotype, cholinesterase levels, cholesterol levels, and urinary chlorpyrifos metabolite levels in both exposed and non-exposed persons. The data analysis and interpretation of this study are currently ongoing.

Discussion: OP Compound Metabolism, Toxicity, and the Role of PON-1

OP Compound Metabolism and Toxicity

Organophosphorous compounds, including chlorpyrifos, are commonly used insecticides worldwide. Their mechanism of action involves phosphorylation and subsequent inhibition of the enzyme acetylcholinesterase (AChE). This action results in impaired ability to hydrolyze acetylcholine, a common neurotransmitter in both the peripheral and central nervous systems (CNS). Acetylcholine excess can then lead to severe toxicity manifesting as a cholinergic crisis or toxidrome, which classically presents as seizures, glandular hypersecretion (lacrimal, sweat, and mucus membranes), vomiting, diarrhea, bradycardia, neuromuscular dysfunction, and other signs and symptoms. When severe, this acute toxicity can be life threatening, with death typically resulting from respiratory failure due to profuse bronchorrhea, bronchospasm, in combination with profound bradycardia.

The United States Environmental Protection Agency, the regulatory agency for domestically distributed pesticides, has recognized the negative impact of certain OPs on children and vulnerable populations. As a consequence, a phaseout for most residential and nonagricultural uses of several OPs (such as chlorpyrifos and diazinon) was instituted in the USA beginning in 2000 [5]. Unfortunately, OPs are still commonly used in farming worldwide due to their low cost and relatively easy accessibility. Although the presentation of acute OP poisoning is well described, much less is known about the clinical and laboratory features of persons with chronic OP exposure and how PON-1 genotype influences this. The latter will be the primary focus of this discussion.

The evaluation of persons with chronic OP exposure and their potential risk for toxicity is challenging. Tests that provide direct measurement of OPs or their metabolites in serum or urine are not readily available. The best laboratory tests for OP exposure would likely be CNS or neuronal AchE, which is the primary target of OP compounds. However, this testing requires obtaining neuronal biopsies, an impractical and expensive procedure to perform on a large scale. Therefore, the most common and readily available tests for OP exposure are plasma and red blood cell (RBC) cholinesterase concentrations. In general, activity of these enzymes is expected to fall with increasing OP exposure as enzymes become deactivated. Plasma cholinesterase activity usually falls first, followed by RBC cholinesterase activity. A fall of greater than 50% in RBC cholinesterase activity when compared to an individual's baseline can result in clinical OP poisoning [6]. Red blood cell and plasma cholinesterase activity, however, has the fundamental limitation of only being a surrogate marker for poisoning. Furthermore, proper interpretation of testing results can be difficult for several reasons. First, individual expression of these enzymes is highly varied and can be affected by many disease states, such as malnutrition or anemia, or drug exposures. In addition, amounts and activity levels of these peripheral blood enzymes may not correlate with the degree of CNS dysfunction, a primary health problem caused by OPs [6].

Due to these limitations, plasma and RBC cholinesterase testing results are poor predictors of the extent and duration of toxicity of OP poisoning. Therefore, further study is needed to enhance understanding of how to appropriately measure OP exposure and toxicity. One such method is by examining the role of PON-1, an enzyme that metabolizes OPs, which is subsequently involved in determining OP toxicity. This is the purpose of the study mentioned above. Although data analysis and interpretation of study results are ongoing and not finished, we provide a brief discussion of human PON-1 [6].

The Role of PON-1 in OP Exposure and Poisoning

Human PON-1 is a calcium-dependent esterase that is synthesized in the liver and transported in the serum along with HDLs. This enzyme serves various functions including preventing the oxidation of low-density lipoproteins, which impairs the development of atherosclerotic plaques, as well as the detoxification of various OP compounds [4]. PON-1 activity is highest in the liver and serum. Specifically, PON-1 hydrolyzes the aryl ester (oxon) group formed after oxidative desulfurization of the OP parent compound by the cytochrome p450 system in the liver. Therefore, PON-1 acts on the metabolites of OP compounds, and not the parent compounds themselves. The name PON-1 is derived from its ability to metabolize paraoxon, the toxic metabolite of the OP compound parathion [4]. PON-1 has also been shown to be active against OP nerve agents, such as sarin, soman, and VX. In fact, PON-1 has been shown to hydrolyze sarin and soman more effectively than it metabolizes the OP metabolite paraoxon [7].

The PON-1 gene has one common polymorphism, which directly impacts biological activity. A substitution involving glutamine (Q) and arginine (R) at position 192 ($PON\text{-}1_{Q192R}$) affects the hydrolytic efficiency of the enzyme. Various OP compounds are metabolized more efficiently by one of the two different PON-1 alloforms. For example, the PON-1 $_{RR\ 192}$ alloform more efficiently hydrolyzes the OP metabolite paraoxon in vitro than the PON-1 $_{QQ\ 192}$ alloform. However, the PON-1 $_{QQ\ 192}$ alloform more efficiently hydrolyzes chlorpyrifos oxon and diazoxon, metabolites of the OP compounds chlorpyrifos and diazinon, respectively [8, 9]. There is a gene-dependent increase in PON-1 activity when the R allele is present, suggesting a biosensor effect during anticholinesterase agent exposure that may upregulate PON-1 activity [10]. PON-1 polymorphisms also seem to influence hydrolytic activity against soman and sarin, with the rate of PON-1 $_{QQ\ 192}$ being 1.5 to 3.5 times that of the $PON\text{-}1_{RR192}$ alloform. Therefore, it is highly likely that individual genetic variation could potentially determine susceptibility to toxicity from OP compounds.

In addition to the genotype, the amount of PON-1 activity is also expected to play a role in modulating the toxicity of some OPs. A polymorphism involving leucine (L) and methionine (M) at position 55 ($PON\text{-}1_{L55M}$) is thought to affect overall PON-1 activity due to varying amounts of protein expression, but does not directly impact PON-1 enzymatic efficiency. The PON-1 $_{MM55}$ genotype is associated with lower PON-1 activity due to lower amounts of mRNA and, therefore, lowers amounts of PON-1 protein synthesis [10]. Interestingly, the genes which code for PON-1 and AchE, the major target for OP compounds, are located in close proximity on chromosome 7q21-22. This suggests that polymorphisms in these adjacent genes can impact each other's expression, which can impact sensitivity to agents that inhibit cholinesterase enzymes [11].

The best evidence thus far for the role of PON-1 activity in modulating OP sensitivity comes from animal experiments. In animals, PON-1 genotype has been shown to directly impact susceptibility to OP toxicity. Animal species with lower PON-1 plasma activity are more sensitive to the toxicity of OPs than species with higher PON-1 activity levels. For example, birds, which have very low or no plasma PON-1 activity, were found to be more sensitive to OP toxicity than rats, which have high activity. Additionally, rats are more sensitive to OP toxicity than rabbits, which have a sevenfold higher plasma PON-1 activity. Experiments in which rats were injected with PON-1 purified from rabbit serum showed that PON-1 has a protective effect against OP toxicity in rats that were administered PON-1 [7].

Studies involving PON-1 knockout mice, which have no PON-1 activity, show the importance of PON-1 in OP metabolism. These animals are extremely sensitive to toxicity from the OPs chlorpyrifos and diazinon. Injection of exogenous purified human PON-1 decreased the sensitivity to diazoxon and chlorpyrifos oxon, and had a protective effect against these compounds, but not against paraoxon. Therefore, it would seem highly likely that other pathways are responsible for paraoxon metabolism. Despite its name, PON-1-based paraoxon metabolism is likely too low to be physiologically relevant, and other pathways are likely responsible for paraoxon detoxification [7]. Animal experiments show that PON-1 activity determines the sensitivity to OP toxicity and that increasing serum PON-1 activity decreases OP toxicity and is protective against some, but not all, OPs in the setting of acute exposure.

Several human studies have investigated PON-1 genotype and OP exposure. A case–control study by Mackness et al. in 2003 examined PON-1 genotype and symptoms of OP poisoning reported in sheep famers exposed to OPs in sheep dip, which is used to protect the sheep from external parasites. They found that individuals with the $PON\text{-}1_{RR192}$ and $PON\text{-}1_{LL55}$ allele were more likely to report symptoms of chronic OP exposure and feel ill, as compared to similarly employed controls who believed themselves to be healthy. This finding is likely due to the fact that individuals expressing the $PON\text{-}1_{RR192}$ and $PON\text{-}1_{LL55}$ alloforms are less efficient at hydrolyzing diazoxon, the major metabolite of diazinon, an OP found in the sheep dip [12].

Another human study by Lee et al. looking at PON-1 genotype and symptoms in 100 farm workers exposed to OPs in South Africa found similar results. Symptoms consistent with chronic OP toxicity were more likely to be reported by pesticide applicators that express $PON\text{-}1_{QQ192}$

(slow metabolizers) compared to pesticide applicators who express PON-1_{RR192} (fast metabolizers). The difference was statistically significant, with 75% of pesticide applicators who are slow metabolizers reporting symptoms compared to only 58.8% of pesticide applicators who are fast metabolizers. In fact, even amongst individuals who were non-pesticide applicators, the prevalence of symptoms was significantly correlated with PON-1 genotype, with 42.9% of slow metabolizers reporting symptoms compared to only 15% of fast metabolizers. This finding was likely because non-applicators are still exposed to OP pesticides through other indirect means. Interestingly, in this study, neither RBC nor plasma cholinesterase levels were statistically significant predictors of chronic cholinergic toxicity [13].

A study performed on 163 OP pesticide handlers in Washington State showed that individuals expressing the PON-1_{QQ192} alloform (slow metabolizers) and those who had lower PON-1 enzyme activity had lower plasma cholinesterase levels relative to OP-exposed agricultural pesticide handlers who expressed PON-1_{RR192} (fast metabolizers). This suggests that PON-1 offers a protective effect against cholinesterase enzyme inhibition in individuals who are fast metabolizers. However, the clinical impact of this finding (symptoms of chronic cholinesterase exposure) was not discussed and needs to be further studied [14].

It is likely that PON-1 genotype plays a role in modulating human OP toxicity. Human studies have shown a correlation between PON-1 genotype and symptoms of OP toxicity in populations highly exposed to OPs. The studies reviewed in this article have shown that fast metabolizers of pesticides, by PON-1, are less likely to report symptoms of OP toxicity than slow metabolizers with similar exposure history. In addition, slow metabolizers who also expressed PON-1_{LL55}, which is associated with decreased PON-1 activity, were found to be the most likely persons to report symptoms of OP toxicity. [12] In addition, PON-1 fast metabolizers have been shown to have higher plasma and RBC cholinesterase levels than their counterparts who are slow metabolizers. [14] Together, these studies suggest a protective effect against OP toxicity in individuals who are PON-1 rapid metabolizers and have higher PON-1 activity. This evidence also suggests that not only is PON-1 protective against OP toxicity, but that exogenous PON-1 may have a potential therapeutic role in protecting individuals exposed to OP compounds (agricultural workers exposed to pesticides or soldiers at risk for exposure to a nerve agent). Thus far, only one study exists which incorporated objective data into its methodology, (cholinesterase testing results), which were determined to be poor markers for OP toxicity [11]. In addition to PON-1 genotype, direct measurement of PON-1 activity and correlation with signs, symptoms, and objective data in real patients may also help to increase understanding of the importance of PON-1 in OP metabolism and toxicity. The ultimate goal would be to better understand how an individual's PON-1 genotype, and resultant PON-1 activity, affects OP metabolism and resultant toxicity. The Centers for Disease Control and Prevention (CDC) study described above seeks to further our understanding of this relationship by incorporating objective measures of exposure, PON-1 genotype and phenotype.

Concluding Thoughts

Organophosphate compounds continue to be used as agricultural pesticides worldwide. They continue to pose a potential health threat to the workers who use them. Current methods of investigating exposure to OPs are limited to only surrogate markers, which screen for exposure, but are not good predictors of consequent health effects. It is becoming apparent that the body's ability to metabolize OPs through the enzyme PON-1 plays a key role in protection from, and detoxification of, these compounds. This metabolism is affected by the genotype as well as the phenotypic expression of this enzyme. The correlation between PON-1 genotype, cholinesterase activity, and clinical toxicity needs to be further evaluated. However, it is very likely that a multifactorial approach, including an assessment of the rapidity of metabolism of specific OPs, in addition to the already existent use of surrogate markers, may be a better method of evaluating exposed patients. The results of the aforementioned CDC study will hopefully clarify some of these issues.

Disclaimer The findings and conclusions in this report are those of the author(s) and do not necessarily represent the official position of the Centers for Disease Control and Prevention/the Agency for Toxic Substances and Disease Registry.

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