Genetic Variability and Sensitivity to Organophosphate Exposures

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& Genome Sciences

Goals of This Presentation

The purpose of this brief presentation is to share with you what we have learned about organophosphate (OP) exposures and the consequences of genetic variability in modulating these exposures. One topic will be the role of plasma paraoxonase (PON1) in protecting against exposure to organophosphorus insecticides, particularly diazinon/diazoxan and chlorpyrifos/chlorpyrifos oxon and the consequences of genetic variability in modulating mixed OP exposures.

PON1 is a high density lipoprotein (HDL) associated enzyme of 354 amino acids that plays a significant role in the detoxication of the highly toxic OP metabolites diazoxan and chlorpyrifos oxon. The role of animal models in understanding the consequences of gene/environment interactions will also be discussed.

Research on biomarkers of exposure, sensitivity and disease will also be discussed.
Biomarkers

• **Biomarkers of susceptibility**
  *Why are some individuals more susceptible than others to a given exposure?*

• **Biomarkers of exposure**
  *How do you know if you have been exposed to a given toxicant (e.g., OP insecticide or tricresyl phosphate)?*

• **Other issues of OP exposure**

• **Biomarker of Parkinson disease**

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**Topics Covered**

• **Genetic variability of OP sensitivity**
  - Main focus will be on chlorpyrifos and diazinon and detoxication via the PON1 pathway
  - Genetic variability of PON1 in human populations
  - Development of an animal model for PON1
  - PON1 variability and mixed exposures
  - Contaminated aircraft cabin air issues

• **Biomarkers of OP exposure**
  - Identification of useful biomarker proteins
  - Characterization of biomarker proteins

• **Biomarker for Parkinson’s disease in males**
The Paraoxonase Family of Genes

PON1 hydrolyzes a number of organophosphorus compounds

The PON proteins can be considered to be modulators of oxidative stress and members of the protein family involved in innate immunity (via their abilities to inactivate quorum sensing factors).

Origin of paraoxonase name

Paraoxon $\xrightarrow{\text{Paraoxonase}}$ Hydrolysis products

- $\text{Diethyl phosphate}$
- $\text{p-Nitrophenol}$
Properties of Human Paraoxonase (PON1)

- **PON1** is an HDL-associated plasma enzyme.
- **PON1** activity is polymorphically distributed in human populations.
- **PON1** metabolizes
  - Toxic organophosphates (insecticides and nerve agents)
  - Oxidized lipids
  - Drugs (activates/inactivates)
  - Microbial quorum sensing factors

Detoxication of OP Insecticides

The commonly used organophosphorus insecticides parathion, chlorpyrifos and diazinon are manufactured as organophosphorothioates. These compounds are very poor inhibitors of cholinesterases. In organisms (target and non-target) the thioate is converted to an oxon form by cytochromes P450. Also, as discussed below, actual exposures include both parent thioate residues as well as the highly toxic oxon forms.

It was thought that mammals could detoxify the oxons as rapidly as they were formed. However, in recent years, it has become apparent that there is considerable variability in different individuals’ plasma paraoxonase (PON1) levels that are controlled developmentally and genetically.

The following slides will elaborate on these factors and the consequence of high vs. low plasma PON1 levels.

An additional concern based on recent findings of researchers from North Carolina State University is that the thioates are suicide substrates for the P450 enzymes that catalyze the oxidative desulfuration of the parent compounds. Of particular interest is the inactivation of cytochromes P450 3A4 and 1A2 that are important in the metabolism of testosterone and estradiol.
Cytochrome P450-Paraoxonase (PON) pathway for Organophosphate Detoxification

Cytochrome P450-Paraoxonase (PON) pathway for Organophosphate Detoxification

Genetic Variability in OP Degrading Enzymes

- Brief historical background of PON1
  - PON1 and OP metabolism
  - Animal models for PON1 function

- Genetic variability in other OP protective enzymes
Population Distributions of PON1 Activities for Different Substrates

Method developed by La Du and Co-workers

Early methods relied on histograms of a single activity to resolve PON1 phenotypes.

\[ \Delta = R/R \]
\[ = Q/R \]
\[ = Q/Q \]

PON1 Status = PON_{192} Genotype & level.
DZOase vs. POase plot completely separates 192 phenotypes (functional genotypes)
PON1 status determined by two-substrate activity analyses provides both the functional PON1-192 genotype as well as plasma PON1 levels that vary by at least 15-fold among individuals.

Problems with Safety Tests

- Most if not all safety tests were carried out with highly pure parent compounds (usually >99%).
- Exposures may contain a significant percentage of highly toxic oxon form of the OP.
- The oxon form is a much more potent inhibitor of cholinesterase than parent compound.
- The genetic and developmental variability of sensitivity to the oxon component is significant.
- Thioates are suicide substrates for P450s.
Concerns about Product Safety Tests

One of the important factors to consider is how the safety tests were carried out with respect to what we now know about the genetically and developmentally variable sensitivity to diazinon/diazoxon exposures.

Safety tests were carried out with highly pure parent compounds, which at the time were the types of tests required by regulatory agencies.

Examples of Purity of Parent Compounds Used for Safety Tests

Safety studies with diazinon used parent compound of 99.5% purity.

For details see: The reconsideration of approvals of the active constituent diazinon, registrations of products containing diazinon and approval of their associated labels. Part 2 Preliminary Review Findings Volume 2 of 2 Technical Reports, June 2006. Australian Pesticides & Veterinary Medicines Authority. Canberra Australia

Safety studies with chlorpyrifos oxon used parent compound of very high purity.

Real life exposures include both parent compound and oxon residues

![Chemical structure of chlorpyrifos and its metabolites](chemical_structure.png)

**Inhibition of brain AChE activity**

<table>
<thead>
<tr>
<th></th>
<th>OP</th>
<th>( K_a ) (M)</th>
<th>( k_h ) (min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpyrifos</td>
<td>((2.64 \pm 1.03) \times 10^{-3})</td>
<td>0.82 ± 0.02</td>
<td>((3.22 \pm 0.48) \times 10^{5})</td>
</tr>
<tr>
<td>Chlorpyrifos oxon</td>
<td>((7.31 \pm 2.52) \times 10^{-3})</td>
<td>2.21 ± 0.04</td>
<td>((3.18 \pm 0.23) \times 10^{5})</td>
</tr>
</tbody>
</table>

**Disassociation constants \((K_a)\) are based on the highest value reported in study. Rate constants \((k_h)\) in micromolar dependence on micromolar substrate.**

Safety tests were carried out with very pure chlorpyrifos


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**Exposures involve direct contact with oxon residues**

<table>
<thead>
<tr>
<th>Pesticide (units)</th>
<th>Oxon (% of Total OP)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Table 1 Oxon levels in total pesticide residues taken from dislodgeable leaf foliar residue and dermal exposure studies</strong></td>
<td></td>
</tr>
<tr>
<td>Pesticide (units)</td>
<td>Oxon (%)</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------</td>
</tr>
<tr>
<td>Ralls et al. (1966)</td>
<td>Diazinon (ppm²)</td>
</tr>
<tr>
<td>Karsouglou and Hopkins (1968)</td>
<td>Diazinon</td>
</tr>
<tr>
<td>Wolfe et al. (1975)</td>
<td>Parathion (ng/cm²)</td>
</tr>
<tr>
<td>Kraus et al. (1977)</td>
<td>Azinphosmethyl (%)</td>
</tr>
<tr>
<td>Nigg et al. (1977)</td>
<td>Ethion (ng/cm²)</td>
</tr>
<tr>
<td>Spear et al. (1977a)</td>
<td>Parathion (ng/cm²)</td>
</tr>
<tr>
<td>Spear et al. (1977b)</td>
<td>Parathion (μg/cm²)</td>
</tr>
<tr>
<td>Spear et al. (1977c)</td>
<td>Parathion (ng/cm²)</td>
</tr>
<tr>
<td>Maddy and Meinders (1987)</td>
<td>Azinphosmethyl (μg)</td>
</tr>
<tr>
<td>Costello et al. (1989)</td>
<td>Malathion (μg/cm²)</td>
</tr>
<tr>
<td>Schneider et al. (1990)</td>
<td>Azinphosmethyl (ng/cm²)</td>
</tr>
<tr>
<td>Spencer et al. (1991)</td>
<td>Azinphosmethyl (μg/cm²)</td>
</tr>
<tr>
<td>McCardy et al. (1994)</td>
<td>Azinphosmethyl</td>
</tr>
</tbody>
</table>

\*Based on the highest value reported in study.
\*Units or values not given in study.
\*ND, none detected.
\*Foliar residue measurement.
\*Dermal monitoring measurement.
Oxon Residues in Exposures

Real-life exposures contain variable levels of highly toxic oxon components. In the study by Ralls et al., the oxon content of the diazinon residues represented 17% of the total residue. In light of what is now known, it makes sense for safety tests to include a range of oxon contents that include percentages of oxon likely to be encountered in actual exposures.


PON1 Status

SNP analyses provide no information about an individual's plasma PON1 levels.

Use of non-OP substrates extend the use of this assay to more laboratories.

Richter et al. Pharmacogenetics 9:745-753
Richter et al. Circ Cardiovasc Genet 1:147-152;
Toxicol Appl Pharmacol 235:1-9
Recently, much better functional two-substrate assays have been developed that separate populations into individuals with specific functional genotypes as will be described below. The assay also provides the level of enzyme present in the plasma of each individual. An important genetic variability in the amino acid present at position 192 of this 355 amino acid protein [glutamine (Q) or arginine (R)] determines whether the PON1 in an individual can hydrolyze paraoxon rapidly or slowly. Since the two so-called alloforms of paraoxonase (PON1-Q192 or PON1-R192) have different properties, this analysis provides the resolution of phenotypes shown in the slide. In the data shown in this slide, DNA analysis was also carried out. There were some discrepancies observed, where the DNA sequence was observed to specify a heterozygous genotype at position 192 (Q/R) whereas the functional assay showed that only one alloform was present in the individual’s plasma. Further studies involving sequencing the entire PON1 genes of these individuals elucidated the reason for the discrepancy. These individuals had PON1 genes that were defective at regions of the gene away from that analyzed by the DNA analysis protocol as noted in the slide. These observations serve to illustrate the accuracy of the functional 2-substrate assay.


### Conversion factors for rates of substrate hydrolysis.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Conversion Factors</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>QQ</td>
<td>AREase$<em>{HS}$ (U/ml) x 172 = DZOase$</em>{phys}$ (U/L)</td>
<td>0.93</td>
</tr>
<tr>
<td>QR</td>
<td>AREase$<em>{HS}$ (U/ml) x 204 = DZOase$</em>{phys}$ (U/L)</td>
<td>0.82</td>
</tr>
<tr>
<td>RR</td>
<td>AREase$<em>{HS}$ (U/ml) x 286 = DZOase$</em>{phys}$ (U/L)</td>
<td>0.87</td>
</tr>
<tr>
<td>QQ</td>
<td>AREase$<em>{HS}$ (U/ml) x 69 = CPDase$</em>{phys}$ (U/L)</td>
<td>0.87</td>
</tr>
<tr>
<td>QR</td>
<td>AREase$<em>{HS}$ (U/ml) x 103 = CPDase$</em>{phys}$ (U/L)</td>
<td>0.88</td>
</tr>
<tr>
<td>RR</td>
<td>AREase$<em>{HS}$ (U/ml) x 159 = CPDase$</em>{phys}$ (U/L)</td>
<td>0.89</td>
</tr>
<tr>
<td>QQ</td>
<td>AREase$<em>{HS}$ (U/ml) x 110 = DZOase$</em>{phys}$ (U/L)</td>
<td>0.84</td>
</tr>
<tr>
<td>QR</td>
<td>AREase$<em>{HS}$ (U/ml) x 100 = DZOase$</em>{phys}$ (U/L)</td>
<td>0.72</td>
</tr>
<tr>
<td>RR</td>
<td>AREase$<em>{HS}$ (U/ml) x 83 = DZOase$</em>{phys}$ (U/L)</td>
<td>0.93</td>
</tr>
<tr>
<td>QQ</td>
<td>AREase$<em>{LS}$ (U/ml) x 45 = CPDase$</em>{phys}$ (U/L)</td>
<td>0.73</td>
</tr>
<tr>
<td>QR</td>
<td>AREase$<em>{LS}$ (U/ml) x 50 = CPDase$</em>{phys}$ (U/L)</td>
<td>0.84</td>
</tr>
<tr>
<td>RR</td>
<td>AREase$<em>{LS}$ (U/ml) x 55 = CPDase$</em>{phys}$ (U/L)</td>
<td>0.92</td>
</tr>
<tr>
<td>QQ</td>
<td>AREase$_{HS}$ (U/ml) x 3.8 = POase (U/L)</td>
<td>0.75</td>
</tr>
<tr>
<td>QR</td>
<td>AREase$_{HS}$ (U/ml) x 15.9 = POase (U/L)</td>
<td>0.50</td>
</tr>
<tr>
<td>RR</td>
<td>AREase$_{HS}$ (U/ml) x 47.6 = POase (U/L)</td>
<td>0.90</td>
</tr>
<tr>
<td>QQ</td>
<td>DZOase$<em>{phys}$ (U/L) x 1.6 = AREase$</em>{HS}$ (U/ml)</td>
<td>0.85</td>
</tr>
<tr>
<td>QR</td>
<td>DZOase$<em>{phys}$ (U/L) x 2.0 = AREase$</em>{HS}$ (U/ml)</td>
<td>0.66</td>
</tr>
<tr>
<td>RR</td>
<td>DZOase$<em>{phys}$ (U/L) x 3.5 = AREase$</em>{HS}$ (U/ml)</td>
<td>0.83</td>
</tr>
<tr>
<td>QQ</td>
<td>DZOase$<em>{phys}$ (U/L) x 0.84 = DZOase$</em>{phys}$ (U/L)</td>
<td>0.90</td>
</tr>
<tr>
<td>QR</td>
<td>DZOase$<em>{phys}$ (U/L) x 1.01 = DZOase$</em>{phys}$ (U/L)</td>
<td>0.91</td>
</tr>
<tr>
<td>RR</td>
<td>DZOase$<em>{phys}$ (U/L) x 0.84 = DZOase$</em>{phys}$ (U/L)</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Conversion coefficient squared
AREase$_{HS}$ = Arylesterase activity measured in buffer and 2M NaCl
DZOase$_{phys}$ = Diazoxonase activity measured under physiological conditions
CPDase$_{phys}$ = Chlorpyrifos oxonase activity measured under physiological conditions
AREase$_{LS}$ = Arylesterase activity measured in buffer
POase = Paraoxonase (PON1) aside (Unconjugated) from Richter et al. (submitted to Circulation: Cardiovascular Genetics)
DZOase$_{phys}$ = Diazoxonase activity measured at 2M NaCl, pH 8.5
Additional ~200 PON1 SNPs discovered by SeattleSNPs (NIEHS Environmental Genome Program)

PON1 is encoded by 9 Exons

Xcr factor Sp1 binding site

Determines Catalytic Efficiency

P90L (low activity)

W194X (premature stop)

L55M (low activity)

124 asp missplice (low activity)

One partial deletion of a glutamine allele detected to date

~200 SNPs

Characterization of all of these SNPs will not allow one to predict plasma PON1 levels. It is necessary to actually measure activity levels.

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

What are the consequences of variability in PON1 status?
What can we learn about PON1 function from rodent models?

What are the consequences of high PON1 levels?

Early studies on the effects of high PON1 levels on resistance to OP exposure involved the injection of purified rabbit PON1 into mice and challenging the mice with a dermal exposure to OPs. The early studies were mostly carried out with chlorpyrifos oxon or chlorpyrifos.

To test whether PON1 protects against OP exposure, we first determined the most suitable route of administration of purified rabbit PON1 into mice. Injection via the iv route was chosen for the experiment on the next slide. At time zero, purified rabbit PON1 was injected into mice via the tail vein and rates of PON1 hydrolysis of chlorpyrifos oxon (CPOase) and paraoxon (POase) were monitored over time.

(Li et al., J Toxicol and Environ Health 1993; 40:337-346).
Plasma levels of PON1 can be increased by injecting purified rabbit PON1

High PON1 levels are protective against exposure to CPO (14 mg/kg)
High levels of PON1 protect against OP exposure

What are the consequences of low PON1 levels?

PON1 activity levels in PON1+/+, PON1 +/-, and PON1/- mice

Role of PON1 in Modulating OP Exposures

The dose response curves for the PON1 deficient mice are dramatically changed for dermal exposure to diazoxon (next slide) but much less so to exposure to the parent compound diazinon (not shown). PON1-/- mice lacking both PON1 genes were killed by dermal exposures (4 mg/kg) that had no measurable inhibition of brain cholinesterase in normal mice as well as by half that dose. Mice exposed to one-fourth the dose (1 mg/kg) of diazoxon exhibited significant signs of OP intoxication. On the other hand, the differences in sensitivity to the parent compound diazinon were less dramatic (following slide). These observations took us back to one of our earlier papers that included a literature survey of the levels of oxon in residues (Yuknavage et al. 1997, slide after next) and re-emphasized the importance of the PON1 genetic variability in modulating exposure to the oxon component as well as a role in detoxifying the parent compound.

Chlorpyrifos oxon is more toxic to PON1\(^{-/-}\) than to PON1\(^{+/+}\) mice

![Graph showing AChE activity in brain with chlorpyrifos oxon concentration on the x-axis and AChE activity on the y-axis.](Shih et al., 1998. Nature 394:284-287)

The Importance of the Mouse Genetic Model

The next slide shows the most surprising result from the series of dermal exposure experiments with the PON1 knockout mice. It was assumed for nearly 50 years that high levels of PON1 would protect against paraoxon toxicity and conversely, low PON1 levels would render individuals sensitive to this OP. As seen in the next slide, we observed no significant differences in paraoxon sensitivity between wild type mice, PON1 hemizygous mice and PON1 knockout mice. The reason for this will become clear in the slide after next.

(Li et al., 2000. Pharmacogenetics, 10:767-779).
**Paraoxon toxicity is not influenced by PON1 status**

**Brain**

![Graph showing AChE Activity (units/g) vs. Paraoxon (mg/kg)](image)


**Catalytic Efficiency, the Key to Understanding the Ability of PON1 to Protect Against OP Exposure**

The next slide provides an explanation for the results seen when the PON1 deficient mice are injected with either purified human PON1-192 alloform (PON1-Q192 or PON1-R192) or saline and exposed dermally to the indicated organophosphates (chlorpyrifos oxon, diazoxon and paraoxon).

PON1-192 alloforms (Q102 or R192) were purified from human plasma from PON1 Status-typed individual human plasma samples. The purified PON1 was injected into the PON1 deficient mice to determine the effectiveness of each alloform to protect against exposure to chlorpyrifos oxon, diazoxon and paraoxon. The degree of protection provided by each alloform was closely related to the catalytic efficiency of the specific alloform for the given OP. PON1-R192 provided better protection against chlorpyrifos oxon exposure, both alloforms protected nearly equally as well against diazoxon exposure with PON1-R192 protecting a bit better and neither protected against paraoxon exposure, in agreement of a lack of increased sensitivity of PON1 null mice to paraoxon exposure.

Thus resistance to diazoxon exposure should be governed primarily by an individual's plasma PON1 levels, whereas resistance to chlorpyrifos oxon exposure depends on plasma PON1 levels as well as position PON1-192 genotype with PON1-R192 providing the best protection.

Catalytic efficiency determines the \textit{in vivo} efficacy of PON1 for detoxifying organophosphates

**Catalytic efficiencies of PON1 192Q and PON1 192R enzymes**

**Chlorpyrifos-oxon Hydrolysis**

<table>
<thead>
<tr>
<th></th>
<th>PON1Q192</th>
<th>PON1R192</th>
</tr>
</thead>
<tbody>
<tr>
<td>Km (mM)</td>
<td>0.84</td>
<td>0.26</td>
</tr>
<tr>
<td>Vmax (units/mg)</td>
<td>82</td>
<td>64</td>
</tr>
<tr>
<td>Vmax/Km</td>
<td>152</td>
<td>256</td>
</tr>
</tbody>
</table>

**Diazoxon Hydrolysis**

<table>
<thead>
<tr>
<th></th>
<th>PON1Q192</th>
<th>PON1R192</th>
</tr>
</thead>
<tbody>
<tr>
<td>Km (mM)</td>
<td>2.98</td>
<td>1.02</td>
</tr>
<tr>
<td>Vmax (units/mg)</td>
<td>222</td>
<td>79</td>
</tr>
<tr>
<td>Vmax/Km</td>
<td>75</td>
<td>77</td>
</tr>
</tbody>
</table>

**Paraoxon Hydrolysis**

<table>
<thead>
<tr>
<th></th>
<th>PON1Q192</th>
<th>PON1R192</th>
</tr>
</thead>
<tbody>
<tr>
<td>Km (mM)</td>
<td>0.81</td>
<td>0.52</td>
</tr>
<tr>
<td>Vmax (units/mg)</td>
<td>0.57</td>
<td>3.26</td>
</tr>
<tr>
<td>Vmax/Km</td>
<td>0.71</td>
<td>6.27</td>
</tr>
</tbody>
</table>

Li et al. 2000. Pharmacogenetics 10:767-780

**Protection afforded PON1\/- mice by injecting human PON1 192Q or PON1 192R enzymes**

Further Development of the Mouse Genetic Model

Further insights into the ability of PON1 to protect against exposure to chlorpyrifos oxon were obtained from studies with "PON1 humanized mice". These mice were generated by Dr. Diana Shih and collaborators at UCLA. Essentially, these mice have their mouse PON1 replaced with human PON1-R192 or PON1-Q192. From the original "founder mice", animals that expressed the same levels of each PON1-192 alloform were chosen for establishing colonies. By choosing animals producing the same levels of each alloform in their plasma, the efficacy in protecting against OP exposure could be tested at any time without having to inject purified human paraoxonase, i.e. they were designed genetically to produce their own human PON1s in the absence of mouse PON1.

The next slide shows that the animals expressing human PON1-R192 were much more resistant to cholinesterase inhibition by chlorpyrifos oxon exposure than PON1 deficient animals with PON1-Q192 expressing animals demonstrating intermediate sensitivity except at high doses, where the PON1-Q191 mice were essentially as sensitive as the PON1 deficient mice. This is a very significant observation, since \~50% of individuals of Northern European origin are homozygous for PON1-Q192. 

Dose Response for Chlorpyrifos oxon exposure of 21d PON1\(^{-/-}\) and PON1 Humanized Mice Expressing

\[ hPON1_{R192} \text{ or } hPON1_{Q192} \]

Important since approximately 50% of many populations are homozygous for PON1\(_{Q192}\).

Cole et al., 2005. Pharmacogenet and Genomics 15:589-598

Other Advantages of the PON1\(^{-/-}\) Mice

PON1 has such a significant impact on the detoxication of the oxons of diazinon and chlorpyrifos that it is difficult to examine the contributions of other enzymes and pathways to the detoxication of these compounds. It will be much easier to examine the contributions of these other enzymes and pathways in the PON1 deficient mice.
Detoxication of OPs in PON1 knockouts

WT mice

PON1

ROH + O−Et

Inactivated Enzymes

WT mice allow for determining the contributions of other pathways to detoxication and metabolism

Other Detoxication Products

P450s

Inactivated Enzymes

P450s

GSH-Xferases

Conjugates

PON1−/− mice allow for determining the contributions of other pathways to detoxication and metabolism

Why are young individuals more sensitive to OP compounds?
PON1 Status in Farm Worker Mothers and Newborns
(Collaboration with UC Berkeley Children's Health Center)

Range of activities predicts:
- 65-fold range in sensitivity to DZO exposure between lowest baby and highest mother.
- 130-160 fold range in sensitivity to CPO exposure between the lowest baby and highest mother (R protection >Q)

Furlong et al., Pharmacogenetics and Genomics. 16:183-190
One important concern – exposure of a developing fetus

**Ongoing Epi Study of WA State Farmworkers**

BChE inhibition after stratification by PON1\textsubscript{192} genotype and level of expression (n=124)

Mean (SD) percent change in BChE activity from baseline*  

<table>
<thead>
<tr>
<th>Genotype \ Genotype</th>
<th>Level of expression\ Level of expression</th>
<th>High (P</th>
<th>Moderate (P</th>
<th>Low (P</th>
</tr>
</thead>
<tbody>
<tr>
<td>R/R</td>
<td>0.53 (6.90) Ref</td>
<td>-0.11 (9.42)</td>
<td>0.841</td>
<td>-8.22 (12.66)</td>
</tr>
<tr>
<td>R/R</td>
<td>-2.06 (8.31) P</td>
<td>-6.17 (9.67)</td>
<td>0.014</td>
<td>-7.58 (13.24)</td>
</tr>
<tr>
<td>Q/R</td>
<td>-9.47 (10.88) P</td>
<td>-7.23 (11.67)</td>
<td>0.046</td>
<td>-12.15 (11.99)</td>
</tr>
</tbody>
</table>

* Test for trend (stratified first by genotype, then by AREase category) was statistically significant (P = 0.002)  
† Based on PON1\textsubscript{192}R genotype, where: high = RR; moderate = QR; and low = QQ  
‡ Based on AREase activity, where: high = >145 U/mL; moderate = 124-145 U/mL; and low = <124 U/mL
What about Mixed Exposures?

![Chemical structures and reactions](image)

Jansen et al. Toxicol Appl Pharmacol. 236:142-153

Other Important PON1 Functions

PON1 activity levels are significantly lower among cases of carotid artery disease (filled symbols) vs. controls (open symbols) of both PON1 192QQ and PON1 192QR genotypes.

A lot of things can happen between the gene encoding PON1 and the final PON1 protein product in the plasma. The high throughput two substrate assay provides the determination of the end result of all of the processes from transcription to the HDL particle and is the method of choice for studies of genetic variation of PON1.

Summary: Consequences of Genetically Variable PON1 Status for OP Exposures

- High levels of PON1_{R192} are protective against CPS/CPO; DZS/DSO exposures
- Low levels of PON1 are a risk factor for CPS/CPO; DZS/DSO exposure
- Position 192 genotype is also important for CPS/CPO exposures
- PON1 status can be important in modulating exposures to mixtures of insecticides.
Variability in Other Enzymes

P450 Metabolism of Chlorpyrifos

Modified from D Dai et al. 2001
P450s Involved in CPS Metabolism (Genetic Variability, Environmental Variability)

- Cyp1A2
- Cyp2B6 (>bioactivation)
- Cyp2C9*1
- Cyp2C19 (>detoxication)
- Cyp3A4(bioactivation>detoxication)
**Interindividual Variability in CYP450s**

Levels can be modulated by environment – diet, drugs

Guengerich FP. Cytochrome P450s and Other Enzymes in Drug Metabolism and Toxicity. *AAPS Journal*. 2006; 8(1): E101-E111.

**Activation/detoxication activities from individual human liver microsomes**

*(determined with the use of specific inhibitors)*

<table>
<thead>
<tr>
<th>Subject</th>
<th>Desulfuration* (Bioactivation)</th>
<th>Dearylation* (Detoxification)</th>
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<tbody>
<tr>
<td>HG006</td>
<td>0.09 ± 0.01a</td>
<td>0.35 ± 0.03a</td>
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<tr>
<td>HG023</td>
<td>0.16 ± 0.01a</td>
<td>0.31 ± 0.04a</td>
</tr>
<tr>
<td>HG042</td>
<td>0.74 ± 0.10b</td>
<td>0.67 ± 0.07ab</td>
</tr>
<tr>
<td>HG043</td>
<td>0.08 ± 0.01a</td>
<td>0.61 ± 0.04ab</td>
</tr>
<tr>
<td>HG112</td>
<td>0.67 ± 0.08b</td>
<td>0.91 ± 0.10b</td>
</tr>
</tbody>
</table>

Modified from J Tang et al. 2001
Thus, both levels of P450 and polymorphisms can influence the outcome of OP exposures.

Other Consequences of PON1 Genetic variability
Interindividual Variability of Carboxylesterase(s)

Another OP Exposure of Interest

Almost all of us spend some time in jet aircraft. There is increasing public awareness of an exposure issue that has been ignored for many years. The jet engine lubricants contain the same molecule that paralyzed thousands of people during prohibition when they consumed ginger extracts (ginger jake) adulterated with tricresyl phosphate. The following short video segments and slides that follow will provide an overview of the problem. The links on the next slide will provide additional information should you want to learn a bit more about this issue.
Discussions at two conferences on cabin air quality (London - 2005; Boeing, Everett, WA - 2004) pointed to the urgent need for developing a method to determine whether or not an individual had been exposed to toxic organophosphorus (OP) compounds (e.g. TCP) during a fume event.

Links to Cabin Air News Stories

- Channel 4 News, London
  http://www.channel4.com/news/articles/world/fears+over+cabin+crew+poisoning/166630
- Channel 7, Australia
  http://au.tv.yahoo.com/sunday-night/video/-/watch/13395216/

There are many additional links to earlier news stories. Contact Prof. Furlong for additional links (clem@u.washington.edu)
Molecules of Interest

Tricresyl phosphate isomers are present in jet engine lubricants

The methyl groups can be: ortho, meta, or para

Why are these isomers of interest? A Very Brief History of TCP Exposures

- **1930**
  - TOCP identified as the cause of paralysis in Ginger Jake Syndrome (Smith et al.)

- **1954**
  - TOCP has to be converted to toxic metabolite (probably in the liver - Aldridge)

- **1961**
  - Structure of toxic metabolite (cyclic saligenin phosphate) determined by John Casida
Tricresyl Phosphate, a Toxicant of Interest

Saligenin cresyl phosphate

Why are some people more sensitive than others?

Oxidized by cytochromes P450

Monoorthocresyl phosphate
Saligenin cyclic cresyl phosphate

Triaryl phosphate metabolism
Biomarkers of Exposure
- to Insecticides
- to Tricresly phosphate isomers

Problems with Urinary metabolites
Urinary metabolites may indicate the OP to which the individual was exposed, however -

There are problems associated with these measurements
1) The ½ life is short and,
2) A false estimate of exposure may result, i.e. the person may have taken up TCP directly, eg.
Problems with Metabolite Measurements

It is not possible to determine if the TCP was taken up directly or resulted from metabolism of CPS.

Proteins whose active sites are covalently attached to organophosphate inhibitors have much longer half-lives (e.g., 11-33 days) than free metabolites in urine or plasma and thereby offer a much broader window in time for assessing quantifying exposures.

Analysis of these modified "biomarker proteins" by mass spectrometry provides a highly sensitive approach for documenting and quantifying exposures.
Modified Protein Biomarkers of Exposure (Porcine carboxylesterase as an example)

Modified Carboxylesterase Peptides (Mike MacCoss)

A.

Swr63
AYFVQFYPFFFPLQVYRQAPFV

Swr272
GDEQGVTIPRQPSAGEVYVVL
TIPSEQGGGEEQV
TIPSEQGGEVTVV
TIPSEQGGEVYVVL

S* = 170 Da shift (aged)

B.

Swr379
SGERIDSQTTADVK
AEEKKASPEANIPRTLIVTFMVDY

Swr384
TIPSEQGGGEEQV
TIPSEQGGEVTVV
TIPSEQGGEVYVVL

Swr473
GDERKRVSSGGDHMDTQFTFLLQGDGFEVK
FURLONG ET AL., 2005

S# = 260 Da shift (not-aged)

M@ = oxidized Met

Furlong et al., 2005

Rapid enrichment of plasma cholinesterase for MS analysis using Immuno Magnetic Bead Separation (IMS)

Provides rapid enrichment of target protein(s)
Ion funnel equipped LTQ (MacCoss lab.)
Increases Sensitivity 6-10X

Another recently found function for all three paraoxonases (PON1, PON2 and PON3) is their ability to inactivate microbial quorum sensing factors, adding this family of enzymes to the systems of innate immunity.
Human and murine paraoxonase 1 are host modulators of *Pseudomonas aeruginosa* quorum-sensing

Egon A. Ozer a,b, Alejandro Pezzulo c, Diana M. Shih d, Carlene Chua c, Clement Furlong e, Aldons J. Luvis f, Everett P. Greenberg g, Joseph Zabner h


Paraoxonase-2 deficiency enhances *Pseudomonas aeruginosa* quorum sensing in murine tracheal epithelia

David A. Stoltz a,b, Egon A. Ozer a,b, Carey J. Ng c,d, Janet M. Yu f, Srinivasa T. Reddy a,b, Aldons J. Luvis e, Noam Bourquard f, Matthew R. Parson e, Joseph Zabner h, and Diana M. Shih d


*Drosophila* are protected from *Pseudomonas aeruginosa* lethality by transgenic expression of paraoxonase-1

David A. Stoltz a, Egon A. Ozer a, Peter J. Tefft f, Martyn Barry a, Lei Liu a, Peter J. Klis h, Thomas O. Moninger h, Matthew R. Parson e, and Joseph Zabner h


These transgenic flies are also resistant to chlorpyrifos!
Interestingly, the last paper by Stoltz et al. adds Drosophila as another animal model for understanding the physiological function of the PON family of enzymes and provides important data on the physiological significance of quorum sensing factor inactivation.

We have looked at biomarkers of sensitivity and exposure. Another variation of the analysis of PON1 status indicates that it may serve as a biomarker for Parkinson's disease (PD) in some male patients. There had been a number of reports linking PON1 genetic variability with PD. We felt that if PD is linked to PON1, a proper analysis of PON1 status of PD patients and controls should reveal the linkage. We expected to find that low PON1 status would be a risk factor for PD as we found for carotid artery disease (Jarvik et al. 2000. Atheroscler. Thromb. Vasc. Biol. 20:2442-2447). However, the analyses appear to pick up a subtle difference in the HDL environment manifest as differences in ratios of rates of hydrolysis of different substrates. The analyses identified 41% of males with PD, but did not distinguish female PD patients from control subjects (manuscript in preparation).

This observation makes sense as the next slide shows that mutations that are associated with PD interfere with mitochondrial function.
Mutations associated with Parkinson Disease

These mutations affect mitochondrial function and the ability to modulate oxidative stress.

What can be done if you are exposed to a high dose of OP? The following slide shows that it is possible to engineer human PON1 for expression in an E. coli bacterial expression system, engineer the PON1 for improved efficiency of OP hydrolysis, purify the recombinant PON1 from the E. coli and inject the purified protein to protect against the toxicity of an OP. This proof of concept showed that human PON1 with a lysine replacing glutamine or arginine at position 192 (increasing the rate of OP inactivation) could be injected into mice whose PON1 had been removed genetically (PON1 knockout mice) resulting in the prevention death from a high dose of dermal diazoxon exposure.
Summary SDS-PAGE of rHuPON1K192 (untagged) purification from E. coli and injection of purified rHuPON1K192 into PON1 knockout mice to rescue them from high dose diazoxon exposure.

The purified, engineered rHuPON1K192 was injected into PON1 knockout mice (no PON1 in their plasma) 10 min following exposure to > 2 LD50’s of diazoxon. Both mice survived for many months.

Summary

- Gene/protein-environment interactions are important in modulating the consequences of OP exposures.
- Genetic and developmental variability are important in determining sensitivity to OPs.
- Protein adducts will provide useful biomarkers of exposure.
- A variation of the PON1 status assay should be useful in diagnosing Parkinson’s disease (PD) or susceptibility for PD.
I hope that this presentation has been useful for you. Additional publications from our research laboratory are listed at the end of this presentation.

There are plans to generate a paraoxonase resource web site that will provide many more references to earlier research and work done in other laboratories. When this site becomes available, a link will be provided.

The next slide lists our many collaborators who have helped explore the different facets of PON1 genetic variability. The following slides include additional references to our studies on organophosphates. If you need to contact me for further information or suggestions for additional research questions, my email address is clem@u.washington.edu and phone is 206-543-1193. My mailing address is: CE Furlong, Div. Medical Genetics, Box 357720, University of Washington, Seattle, WA 98195-7720.

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<tr>
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<td>GENETICS, purification &amp; expression</td>
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| Genomics |
| D Nickerson |
| C Carlson |
| M Rieder |
| G Jarvik |

| Parkinson’s Studies |
| Harvey Checkoway |
| Paola Costa-Mallen |
| Fred Farin |
| Samir Kelada |
| Gary Franklin |
| ALS – R Brown, A-M Wills |

| Cardiovascular studies |
| T Bacus |
| G Jarvik, T Hatsukama, J Ranchalis, R Richter |

| UCLA |
| Pen1- and transgenic mice |
| AJ Lusis |
| DM Shih |
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| UC Berkeley |
| Mother/Infant Study |
| B Eskenazi |
| N Holland |
| A Bradman |

| PNNL, Batelle |
| PBPK/PD Modeling |
| C Timchalk |

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The contents of this presentation are solely the responsibility of the presenter and do not necessarily represent official views of the NIH or EPA.
References from our laboratory


References from our laboratory continued


References from our laboratory continued

References from our laboratory continued

References from our laboratory continued

- Richter RJ, Jarvik GP, Furlong CE. Paraoxonase 1 (PON1) Status as a Risk Factor for Disease or Exposure. Submitted for inclusion in the volume “Paraoxonases in Inflammation, Infection and Toxicology”. Humana Press.