Research Brief 190:
Determining Susceptibility to Environmentally-induced Neurotoxicity

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Superfund Research Program
http://www.niehs.nih.gov/srp/

Background

For over 20 years, Dr. Clement Furlong's research group at the University of Washington has focused on the role of the paraoxonase 1 gene (PON1) in metabolism of organophosphate (OP) pesticides. With funding from NIEHS and the Superfund Research Program, this group has made significant contributions:

Organophosphate pesticides can be absorbed via inhalation, ingestion, and dermal routes. OPs are potent nerve agents, functioning by inhibiting the action of acetylcholinesterase in nerve cells.

- Isolated and cloned human and rabbit PON1, and identified a polymorphism at position 192 in the coding sequence of the gene that plays a critical role in PON1 catalytic efficiency.
- Developed the concept of "PON1 status". This assay uses the characterization of genotype ($PON1_{R192}$ vs. $PON1_{Q192}$ isoform) and the phenotype (level of PON1 in the plasma) to determine how rapidly an individual will metabolize a toxicant. "PON1 status" is used by laboratories around the world to investigate risk factors for disease or exposures.
- Developed an animal model to investigate the importance of PON1 variability in determining sensitivity to OP exposures. This study identified the potential for the use of PON1 to protect against the acute toxicity of specific OP exposures.
- Showed that PON1 status is important in modulating the effects of exposures to mixtures of OP pesticides.
- Conducted studies of PON1 genetic variability in Parkinson's disease patients, yielding a new approach for identifying disease risk in men. Identifying individuals at risk may allow for preventative therapy prior to symptom onset.

In current studies, Dr. Furlong is working to identify biomarkers of exposure and susceptibility to OP and related compounds.

Advances

The Furlong research group has successfully identified biomarkers for two quite different OP toxicants with very different exposure scenarios, and has optimized rapid protein target enrichment and mass spectrometric analytical protocols for each.

Rather than follow the usual approach of investigating contaminant metabolites as biomarkers, Dr. Furlong's strategy is to focus on **proteins** that are modified by OP exposure because: (1) modified proteins have longer half-lives than OP compounds or their metabolites, providing a longer window of opportunity for detection, and (2) detection of a metabolite does not provide information about whether an individual was exposed to the parent compound or to the metabolite.

PON1 status as a biomarker of susceptibility to OP pesticides: Plasma cholinesterase (BChE) inhibition is a well-established biomarker of early OP-related biological effects. In collaboration with the Washington State Department of Labor and Industry, Dr. Furlong's group examined the relationship between BChE inhibition and PON1 status following pesticide handlers' exposure to OP pesticides. This large study, with extensive documentation of individual exposures, established that low PON1 status ($PON1_{Q192}$ isoform and low levels of PON1 in the plasma) is a determinant of levels of BChE inhibition among OP-exposed agriculture workers. This finding suggests that PON1 status should be considered as a biomarker of susceptibility to OP-related effects and that regulatory risk assessors should consider differences in PON1-related sensitivity to OP insecticides when characterizing inter-individual variability in risk related to OP exposure.

Biomarkers of exposure to TCP: Air crew members have reported neurological symptoms such as dizziness, nausea, blurred vision, tremors and short-term memory issues. These symptoms, "aerotoxic syndrome", are suspected to be related to incidents of cabin air contamination with engine fumes containing tricresyl phosphate (TCP), a common additive in engine lubricants and hydraulic fluids. Incident reports suggest that some individuals are significantly more sensitive than others to the neurotoxic exposures, and Dr. Furlong applied his expertise with OP-related neurotoxicity to search for modified proteins as biomarkers of exposure to TCP. His research group used mass spectroscopy to examine adducts formed on BChE following reaction with CBDP (TCP is activated to CBDP *in vivo*). They focused on regions of BChE known to react with OP pesticides and, using samples, of CBDP spiked plasma identified phosphoserine adducts on Ser198 that can serve as biomarkers of exposure. This diagnostic tool will contribute greatly to epidemiologic studies on aerotoxic syndrome as well as pesticide exposures among agricultural workers.

Significance

Dr. Furlong's research is contributing to our understanding of the genetic and developmental variability in sensitivity to the toxicity of OP pesticides and reducing the uncertainty in risk assessment. He actively interacts with environmental and health agencies at both the state, federal, and international level to communicate his findings to support policy decisions and programs that have direct impacts on public health. Dr. Furlong's work was considered in EPA's decisions to restrict use of chlorpyrifos and diazanon, and the State of Washington has incorporated his findings on genetic and developmental variability in resistance to OP pesticides into its pesticide training program.

The biomarker protocols that his research group had developed are useful not only for OP pesticides, but to evaluate exposures to nerve agents (e.g., Sarin) and to TCP exposures in aircraft. Dr. Furlong's work to develop assays for Parkinson's disease may be useful for early diagnosis and for monitoring the efficacy of therapeutic protocols. The PON1 status analysis has also proven useful in identifying low PON1 status as a risk for carotid artery disease.

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To learn more about this research, please refer to the following sources:

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