Research Brief 165: PON1 as a Potential Treatment for Organophosphate Poisoning

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Background:

The World Health Organization estimates that ~3 million pesticide poisonings occur each year worldwide, resulting in 220,000 deaths.

The paraoxonase enzymes (PON1, PON2, and PON3) play important roles in gene-environment interactions, drug metabolism, and susceptibility to vascular and infectious disease. Since 2000, SBRP-funded researchers at the University of Washington (http://tools.niehs.nih.gov/sbrp/programs/Program_detail.cfm?Project_ID=P42ES4696/) (UW) have conducted a series of investigations to determine: 1) the role of PON1 in modulating the toxicity of mixtures of organophosphate (OP) compounds, and 2) the direct impacts of PON1 on individuals' susceptibility to pesticide poisoning and cardiovascular diseases.

Drs. Lucio Costa and Clement Furlong began with basic biomolecular studies to increase our understanding of PON1 function and modulation. They developed a strategy to characterize both the PON1 genotype (polymorphisms at position 192 in the coding sequence) and phenotype (plasma levels of PON1) of an individual, allowing them to establish an accurate measure of total PON1 activity. Drs. Costa and Furlong have used this "PON1 Status" biomarker in studies to examine the association of PON1 and an individual's susceptibility to environmentally-induced diseases, including Parkinson's Disease and atherosclerosis.

Advances:

Because PON1 can inactivate a wide range of OPs, including metabolites of the pesticides chlorpyrifos, diazionon and parathion, and the nerve agents sarin, soman, and VX, the researchers proposed that PON1 might be an excellent candidate for treatment of OP exposures. They demonstrated PON1's therapeutic potential by injecting PON1 into control rats and mice and then into PON1 knockout mice (i.e., animals completely devoid ofplasma PON1 activity) both before and after OP exposure. The injected PON1 increased or restored PON1 levels in the knockout mice and conferred resistance to the highly toxic metabolites of chlorpyrifos and diazionon exposure, but not to parathion exposure. This work showed the importance of PON1 Status by revealing that the two different PON1 192 variants have different catalytic efficiencies, which, together with plasma PON1 level, determine whether PON1 can protect against a specific OP exposure.

In work published on-line (August 18, 2008) in the Proceedings of the National Academy of Sciences of the United States of America (http://www.pnas.org/), Dr. Furlong presents the results of work aimed at purifying native and engineered PON1 (rHuPON1) from E. coli, and characterizing the potential of PON1 as a therapeutic agent to treat OP exposures.

The UW team injected the PON1 variants into PON1 knockout mice and found that:

- rHuPON1 was not toxic.
- rHuPON1 had a half-life comparable to the native PON1, with plasma levels peaking ~8 hours after injection and persisting for 48 hours.
- rHuPON1 protected the PON1-deficient mice from OP doses up to three times the median lethal dose value when injected before or after exposure.

While conducting this research, the UW team also resolved a procedural challenge that had hampered other laboratory groups who were unable to express and purify active rHuPON1 from bacterial cells. They learned that successful expression of active rHuPON1 depends on the media used and on the temperature for growth. The E. coli system did not produce active rHuPON1 when grown at temperatures of 37°C or above.

Significance:

This work is the first successful expression and purification of enzymatically active, native and engineered recombinant human PON1 from E. coli. Moreover, these experiments clearly demonstrate the therapeutic potential of E. coli-expressed engineered rHuPON1. Dr. Furlong believes that variants with even higher catalytic efficiencies should be attainable and could prove useful for treating OP pesticide and nerve agent exposures.

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

Furlong, Clement E., Rebecca J. Richter, T.B. Cole, S.S. Park, S.M. Suzuki, and R.C. Stevens. 2008. Engineered recombinant human paraoxonase 1 (rHuPON1) purified from Escherichia coli protects against organophosphate poisoning. Proceedings of the National Academy of Sciences of the United States of America (PNAS).105(3):12780-12784.
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